NEW SERIES VOL. 23 NO. 89 JANUARY 1959

ANNALS OF BOTANY

EDITED BY

W. H. PEARSALL

ASSISTED BY

F. G. GREGORY

T. M. HARRIS

C. T. INGOLD

K. MATHER

OXFORD: AT THE CLARENDON PRESS

Price 25s. net

CONTENTS

NOTICE TO CONTRIBUTORS

Contributors should address their papers to Prof. W. H. PEARSALL, Department of Botany, University College, London, W.C. 1.

Papers sent for publication *should be typewritten. They should include a short abstract and should conclude with a summary of the contents.* In view of the increasing numbers of manuscripts submitted, the Editors desire to impress upon contributors the importance of conciseness. Bodies of quantitative data, too extensive for complete publication, should be summarized for discussion and the originals deposited in the Archives established for this purpose in the British Museum (Natural History), South Kensington, S.W. 7. The Editors suggest that the longer papers should not exceed 10,000 words. Contributors will receive one proof in page. An allowance at the rate of ten shillings per sheet of sixteen pages is made for alterations in the proof (printers' errors excepted), and contributors will be responsible for any excess.

ILLUSTRATIONS. These where possible should be suitable for reproduction as line-blocks in the text. Where half-tone plates are required the figures should be planned so as to fill properly a 4to or an 8vo plate. The maximum space available for figures in a 4to plate is $8\frac{1}{4} \times 11\frac{1}{4}$ inches, in an 8vo plate $8\frac{1}{4} \times 5\frac{1}{4}$ inches. The lettering of figures, whether text-figures or those of plates, should be in pencil.

The Journal is published four times yearly by the publishers, Oxford University Press, Amen House, Warwick Square, London, E.C. 4, to whom subscriptions and all communications, other than editorial, should be addressed.

The subscription price for four numbers is 65*s.* (foreign postage, 1*s.* 6*d.* extra), single copy 25*s.* net. Cloth cases may be obtained, price 7*s.* 6*d.*

FIRST SERIES (VOLUMES 1–50)

Orders and inquiries, other than those from U.S.A., for copies of the first series (Volumes 1–50) of the Annals of Botany should be addressed to The Assistant Treasurer, Annals of Botany Company, Botany School, Cambridge, England. Messrs. J. S. Canner & Co., 46 Millmont Street, Boston 19, Mass., are the sole distributors in the U.S.A. and all American inquiries should be addressed to them.

Stocks of Volumes 1, 2, 19–25, 28, 30–34, 36–40 *are exhausted.* Most of the early volumes are in sheet form, unbound; and in a few cases the volumes are slightly imperfect.

The prices of the back numbers, including packing and postage, are £2. 10*s.* 0*d.* a volume, whether in the form of bound parts or separate sheets (half price if imperfect); 20*s.* a single part (bound); 10*s.* a single part (in sheets). Indexes at usual published prices.

Cheques, other than those from U.S.A., should be made payable to The Annals of Botany Company and sent to The Assistant Treasurer.

The publishers are signatories to the Fair Copying Declaration in respect of this journal. Details of the Declaration may be obtained from the office of the Royal Society upon application.

THE NEW PHYTOLOGIST

FOUNDED BY SIR ARTHUR TANSLEY IN 1902

EDITED BY

A. R. CLAPHAM H. GODWIN W. O. JAMES

October 1958 CONTENTS Vol. 57, No. 3

The Effects of Aqueous Extracts of Red Beet Root on Salt Accumulation and Respiration of Discs of Red Beet Root

BY

J. E. DALE[1] AND J. F. SUTCLIFFE

(*Dept. of Botany, King's College, London*)

With Six Figures in the Text

ABSTRACT

An account is given of the preparation of aqueous extracts of red beet root which are shown to stimulate potassium uptake in beet discs washed for a short period, but inhibit potassium uptake in discs washed for four days or more. Analysis of extracts showed them to contain organic anions (especially citrate and malate) which affect both the metabolic phase of potassium uptake and respiration of the tissue. The effects of extracts and organic acids on uptake of manganese by beet discs is described and compared with effects on potassium absorption. The results are discussed with respect to current theories of salt accumulation and in relation to the hypothesis relating an inhibitor of salt accumulation to the lag phase of ion uptake by beet discs.

INTRODUCTION

IT is well known that when discs of plant tissues are placed in solutions of mineral salts there is, first, a phase of rapid absorption of cations which is complete within a few hours. It is thought that this rapid uptake is of a physical nature and explicable in terms of either the establishment of Donnan equilibria or adsorption on charged cellular constituents. Following this phase of uptake, but not always immediately, is a phase of slower absorption which continues over a prolonged period and is closely associated with aerobic metabolism. There is no significant physical uptake of anions; these may be accumulated immediately on immersion in aerated salt solution or, more usually, after a delay of varying duration.

Stiles and Dent (1946) showed that in red beet root tissue the two types of uptake are usually separated by a 'lag phase' during which time little or no cations or anions are absorbed. These authors showed, as had Asprey (1933) for potato tissue, that the lag period can be reduced if the tissue is pretreated by washing in running tap-water. It was concluded by Stiles and Dent that 'the lag period occurs while metabolic activity is rising to a level necessary for accumulation to take place'.

Rees (1949) found that when discs of red beet were washed in water from which air had been excluded, uptake of both ionic species from a solution of manganese chloride was enhanced. Bubbling the washing solution with air or even nitrogen further increased the uptake of both ions. Rees also found

[1] Present address: *Cotton Research Station, Namulonge, P.O. Box 884, Kampala, Uganda.*

[Annals of Botany, N.S. Vol. 23, No. 89, 1959.]

that treatment of discs with moisture-saturated air had a similar effect to washing, in reducing the lag period. Treatment with moisture-saturated nitrogen had a much less marked effect. Rees suggested that treatment with air and water separately, or in combination, contributes to a reduction of the lag phase.

Later, Rees and Skelding (1950) and Skelding and Rees (1952) reported the extraction from dormant red beet roots of a substance or complex of substances which would inhibit the uptake of manganese by washed beet discs supplied with manganese chloride. It was claimed that the lag phase of uptake is caused by the presence of such an inhibitor which is leached out of the tissue or, alternatively, oxidized by an unknown mechanism, during the process of washing in aerated water. Skelding and Rees considered two possible explanations for the mode of action of the inhibitor. The first of these was that 'the primary effect of the inhibitor is to lower the general level of metabolism of the cell'. On this hypothesis it might be expected that the inhibitor preparations would lower the rate of respiration of beet discs. Skelding and Rees, however, reported that extracts had no respiratory effects. The alternative explanation was that the inhibitor reduces the permeability of the cytoplasm of beet cells to salts and water. The fact that respiration was apparently unaffected under these conditions was explained by the postulation that respiration is 'limited by internal factors rather than the permeability of the membranes to oxygen and carbon dioxide'. Dale and Sutcliffe (1956), in contrast to Skelding and Rees, found that beet extracts had a large stimulatory effect on respiration of beet tissue.

Sutcliffe (1954) presented evidence from studies on the exchange of potassium by beet discs washed for varying lengths of time, which led him to postulate the formation or activation of carrier molecules participating in the accumulation process. He suggested that the lag phase is a period during which new carriers are either synthesized or made available, and as a result of this a capacity for accumulation is built up. Skelding and Rees's initial assumption would seem to be that the material has, from the time of cutting, a propensity for accumulation and that during the washing period an accumulation inhibitor is removed. These two hypotheses are not necessarily mutually exclusive in that the breakdown of an inhibitor may render available for the transport mechanism carrier molecules at sites which were previously blocked.

This paper describes attempts to discover the nature and role of Skelding and Rees's inhibitor in relation to the salt accumulation mechanism of red beet root cells.

Materials and Methods

Disc preparation. Discs $0{\cdot}8 \times 7{\cdot}5$ mm. were cut from roots of the red beet varieties Crimson Globe and Detroit Dark Red. The methods for preparing and washing the discs have been fully described by Sutcliffe (1952). In the present experiments discs were pretreated by frequent changes of distilled water for periods from 2 hours to 6 days.

Salt accumulation. From the work of Skelding and Rees there was reason to suppose that inhibitory extracts from beet root would have a general effect on cation uptake, and in our experiments effects on potassium, manganese, and, in a few experiments, sodium uptake, were determined. Potassium and sodium were determined using an EEL flame photometer, and manganese was estimated by the periodate oxidation method (Vogel, 1951).

In some experiments, determinations of chloride uptake were made using the electrometric method of Furman and Low (1935). Where citrate uptake was studied the modified pentabromacetone oxidation method due to McArdle (1955) was used for the estimation of citrate.

The experimental techniques used in studying potassium and sodium uptake have been described by Sutcliffe (1952). In our experiments 30 standard discs (fresh weight about 1·1 g.) were placed in 4 ml. of solution and shaken in small flasks for periods up to 24 hours. The experimental solution was analysed either by removing an aliquot at intervals throughout the experiment (in which case fresh medium was added to make up to the original volume) or at the end of the experimental period.

When manganese uptake was studied a different apparatus was used. This comprised a metal stand holding eighteen 10 × 1½ in. boiling-tubes each connected to a small air-pump by means of a manifold unit. Stand and tubes stood in a water bath thermostatically controlled at 25° C. The tubes contained either 50 ml. or 60 ml. of experimental solution in which were put 39 standard beet discs. The solutions were aerated continuously and the flow of air kept the discs circulating in the solution.

Respiration. In a number of experiments the respiration rate of samples of discs in 4 ml. of experimental medium was determined using standard Warburg manometric techniques.

Preparation of inhibitor extracts. Extracts were obtained from beet root discs (35 × 0·8 mm) by one of two methods. In the first of these 75 g. of tissue were left standing in 100 ml. of water in a 10 × 1½ in. boiling-tube for 24 hours at room temperature (18° to 22° C.). In the second method a similar weight of tissue was extracted in 100 ml. of boiling water for 90 seconds. After extraction the liquid was decanted and 'standardized' in the following way:

(*a*) *pH*—this was adjusted to a value of 6·0 by the addition of very small amounts of 0·1 N. KOH.

(*b*) *K content*—the concentration of this ion was adjusted to 0·02 M. by the addition of small amounts of either a molar solution of KCl or distilled water.

(*c*) *Na content*—the concentration of this ion in the extract was measured using a flame photometer, and small amounts of sodium chloride were added to the control solution of potassium chloride to bring the concentration of sodium to the level of that in the extract. Except in some of the experiments on manganese uptake the sodium concentration in the test solutions never exceeded 0·001 M. and was generally much lower.

Analytical procedures. Details of analytical procedures involving ion

exchange resins and paper chromatography are discussed in relevant parts of the text.

Results

Preliminary Experiments

The first series of experiments was designed to compare the relative effectiveness of extracts prepared by the two methods already outlined in inhibiting potassium uptake by washed discs. Using discs previously washed for 4 days

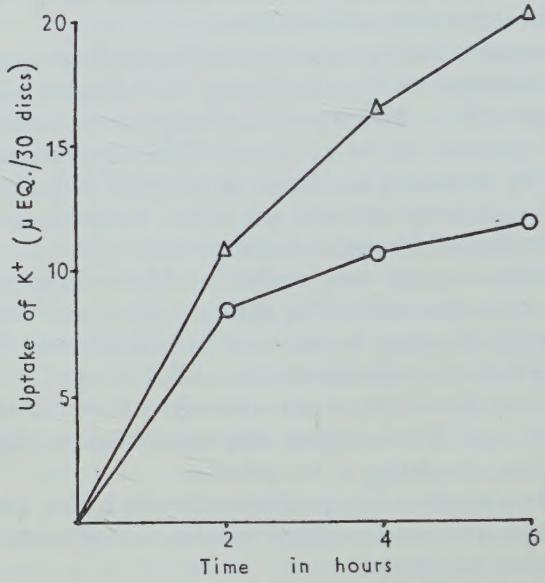


Fig. 1. Uptake of potassium by beet discs in 6 hours at 25° C. from 0·02 M. KCl (△) and beet extract (○).

in water it was found that in a 6-hour period 'standing type' extracts caused a mean inhibition of potassium uptake of 32·6±3·7 per cent. In a parallel series of experiments 'heat type' extracts gave an inhibition of 31·5±4·1 per cent. Clearly both types of extraction procedure were equally effective in providing a solution of the inhibitory agent. It was also found in these experiments that in no case did the inhibition of potassium uptake exceed 46 per cent. According to unpublished observations of Sutcliffe, from 30–50 per cent. of the uptake by 4-day washed discs in a 6-hour period is due to metabolic accumulation. It was of interest to discover whether the inhibitor acted on physical or metabolic uptake, or upon both, and the next experiments were designed to establish this point.

The uptake of potassium from solutions containing KCl or inhibitor preparation was followed by analysing the medium at 2-hourly intervals over a period of 6 hours, to determine at which stage the inhibitor was having its maximum effect. It is clear from the results (Fig. 1) that the greatest inhibi-

tion of uptake occurred in the final period (4–6 hours), when metabolic absorption predominates, and the least inhibition in the period from 0–2 hours, during which time physical uptake constitutes the major absorption mechanism. That metabolic uptake was chiefly affected by the inhibitor was confirmed in another experiment in which discs were allowed to take up salt from experimental solutions kept at 1·5° and 25° C. (Table 1). The results show that the inhibitor only affected uptake at the higher temperature and there was no indication that physical uptake was affected.

TABLE 1

Potassium Uptake (as μeq./g. fresh wt.) in 6 Hours by Beet Discs placed in Solutions of KCl and Beet Extract

Solution	Temperature	Uptake μeq.	Inhibition
0·02 M. KCl	25° C.	25·2±0·97	30%
Extract	25° C.	17·6±0·70	
0·02 M. KCl	1·5° C.	10·7±0·12	n.s.
Extract	1·5° C.	10·0±0·16	

The Inhibitor and the Lag Phase

Rees and Skelding (1950) claimed that extracts prepared from young, actively growing beet did not inhibit manganese uptake by beet discs and suggested that such roots lack the postulated inhibitor. Middleton (1954) showed that freshly cut discs from actively growing beet had a rate of uptake considerably lower than that of similar, but well-washed material. This suggested a lag phase in young beet comparable with that in dormant tissue and we have confirmed this in an experiment in which discs cut from actively growing and dormant beets were washed in water for varying periods prior to a 6-hour period of uptake in 0·02 M. KCl or beet extracts (Table 2).

TABLE 2

Potassium Accumulation (as μeq./g. fresh wt.) by Discs washed for Varying Periods in Distilled Water and then placed for 6 Hours in (a) *Beet Extract and* (b) 0·02 *M. KCl*

1. Extract made from actively growing material tested on discs cut from the same material. Discs cut 18/8/54

Washing period in hours	2	48	96	144	192
0·02 M. KCl	8·5±0·26	11·4±0	13·0±0·27	15·4±0·37	22·1±0
Extract	9·8±0·49	15·4±0·41	14·2±0·21	16·0±0·57	18·4±0·30
Effect of extract	n.s.	+40%	n.s.	n.s.	−17%

2. Extract made from dormant material tested on discs cut from the same material. Discs cut 15/11/54

Washing period in hours	2	48	96	144	192
0·02 M. KCl	9·7±0·27	10·2±0·34	15·2±0·61	20·5±0·11	26·7±0·15
Extract	9·8±0·48	16·7±0·21	16·0±0·35	18·1±0·20	19·6±0·27
Effect of extract	n.s.	+64%	n.s.	−12%	−27%

The rather startling fact that emerged from this experiment was that discs washed for only a short time (48 hours) showed an enhanced uptake of potassium when treated with extracts. This phenomenon was not confined to actively growing material but was also shown by dormant tissue. Other results (Dale, 1956) showed that extracts from actively growing and dormant material had similar effects when applied to discs cut from either actively growing or dormant material. The stimulation of uptake is therefore related to the length of washing pretreatment rather than to differences between the two types of discs or extracts used. It must be concluded that extracts from young growing tissue contain an inhibitory factor similar to that extractable from dormant tissue.

That extracts could stimulate salt uptake by tissues in the lag phase seriously detracts from Skelding and Rees's belief that the inhibitor and lag phase are causally related. In view of this an experiment was performed to determine whether or not the inhibitor was present in washed discs which themselves had a strong capacity for salt accumulation. This was done as follows: 750 beet discs ($11{\cdot}5 \times 1$ mm.) were cut from dormant material and washed in the usual manner for 6 days (i.e. until the lag period might be expected to have ended), after which 6 samples of 10 discs were removed and washed for a further 24 hours. The remaining discs were extracted by the 'standing procedure' for a period of 24 hours. The resulting extract was standardized and tested on the samples of discs previously removed. In this way an extract was made from, and tested, upon similar discs. The results showed that discs in 0·02 M. KCl took up $28{\cdot}0 \pm 0{\cdot}54$ μeq.K/g. fresh wt./6 hours, whilst those in extract took up only $15{\cdot}4 \pm 0{\cdot}39$ μeq.K/g. fresh wt./6 hours. This represents a 45 per cent. inhibition of uptake by the extract. Results of the same order were obtained in another experiment in which discs washed for 7 days were subjected to a 'heat type' extraction. Hence the tests showed conclusively that an inhibiting substance can be extracted from discs which have a strong capacity for salt accumulation.

The Analysis of Extracts

It now became clear that some form of purification of extracts was necessary if the role of the inhibitor in disc metabolism was to be effectively studied. An attempt was made to do this by treatment of the beet extract with either the cation exchange resin Amberlite IR 120 (hydrogen form) or the anion exchange resin Deacidite FF (chloride form). These resins were regenerated by standard methods and used in the form of columns. After passage of the extract the resulting effluent was titrated to pH $6{\cdot}0 \pm 0{\cdot}1$ with either 0·1 N. HCl or 0·1 N. KOH, according to whether anions or cations had been exchanged. Control untreated extracts, and extracts treated with either resin, were tested for respiratory activity and for their effect on salt uptake. The results are given in Table 3.

It will be observed that extracts treated with resins of either type caused only a slight reduction in the stimulation of oxygen uptake. On the other

hand, treatment of extracts with anion exchange resins completely removed all inhibitory effect on potassium uptake, whereas treatment with cation exchange resins had no significant effect. These results were obtained consistently in a number of experiments and it was concluded that the salt accumulation inhibitor was probably anionic, whereas the respiratory factor was of a non-ionic nature. Dale (1956) showed that beet extracts contained up to 2 per cent. of sugars and both he and Middleton (1956) demonstrated that

TABLE 3

Potassium Uptake (as μeq./g.fresh wt./6 hrs.) and Oxygen Absorption (μl. O_2/g. fresh wt./hr.) by Washed Beet Discs placed in (a) 0·02 *M. KCl*, (b) *Beet Extracts*, (c) *Beet Extracts treated with Ion Exchange Resins*

Treatment	K uptake	% inhibition	O_2 uptake	% stimulation
Exp. 93				
0·02 M. KCl . .	25·8±0·10	—	114	—
Extract, control . .	16·7±0	35	153	34
Cation-free extracts . . [Treated with Amberlite IR120 (H)]	18·6±0	28	143	25
Exp. 56				
0·02 M. KCl . . .	25·5±0·59	—	161	—
Extract, control . .	15·7±0·40	38	208	29
Anion-free extract . . [Treated with Deacidite FF (Cl)]	24·0±0·82	n.s.	193	20

glucose caused a large stimulation of oxygen uptake by beet discs. It is suggested that the sugar present is responsible for the greater part of the effect of extract on the respiration rate.

Further experiments showed that the stimulation of salt uptake by extracts tested on beet discs washed for a short time was also anionic. These results were confirmed by a second technique involving the ether extraction of acidified extracts, as follows: Extracts from both active and dormant tissue were acidified to pH 1·0 with 3 N. H_2SO_4 and extracted for 20 hours with peroxide-free ether in a Kutscher–Steudel apparatus. The ether-soluble substances were taken up in water, neutralized with KOH, and made up to their original concentration, adding, if necessary, small amounts of KCl to bring the potassium content up to 0·02 M. The acidified ether-insoluble residual solution was treated with barium hydroxide and solid barium carbonate to remove all sulphate. This preparation (mother liquor), was then standardized in the usual way. The effects of these preparations on salt uptake and on respiration are shown in Table 4 together with the results from a similar experiment in which the extract was not acidified before extraction.

It is clear that the inhibitory factor can be removed by ether from an acid solution but not from a neutral one. From the respiration data it is apparent that a small respiratory effect is associated with that ether extract which affects salt accumulation. It was shown by similar methods, using discs washed for

1 day only, that the stimulatory substance was also ether soluble, but in this case there was no concurrent respiratory effect.

It would appear that ether extraction of acid solutions gives an almost complete recovery of the substances affecting salt uptake and further analyses were carried out by means of conventional paper chromatography techniques on such extracts. Ether soluble fractions were prepared from 50 ml. of beet extract and taken up in 1 ml. of water. About 2·5 μl. of this solution was

TABLE 4

Potassium Uptake (as μeq./g. fresh wt./6 hrs.) and Oxygen Absorption (μl. O_2/g. fresh wt./hr.) by Beet Discs (washed previously for 4 days in distilled water) from 0·02 M. KCl and Various Extracts from Beet Discs

Treatment	K Uptake	% inhibition	O_2 absorption
Exp. 94			
0·02 M. KCl	30·5±0·22	—	129
Water extract	18·9±0	41	290
Acid ether-extract	19·7±0·30	39	150
Mother liquor after acid ether-extraction	30·1±0·27	n.s.	241
Exp. 96			
0·02 M. KCl	30·0±0·71	—	158
Water extract	19·3±0·35	36	321
Neutral ether-extract	28·7±0·03	n.s.	162
Mother liquor after neutral ether-extraction	18·1±0	40	286

spotted on to Whatman No. 1 filter paper and developed as a descending chromatogram in one of the following solvents:

(*a*) Chloroform 66 parts.
95 per cent. ethanol 33 parts.
90 per cent. formic acid 2 parts (Stark, Goodban, and Owens, 1951).

(*b*) Chloroform 80 parts.
t.-amyl alcohol 80 parts.
Water 30 parts.
90 per cent. formic acid 30 parts (Bentley, 1953).

(*c*) Iso-propanol 60 parts.
0·88 ammonia 20 parts.
Water 20 parts (Isherwood and Hanes, 1953).

Using this technique it was demonstrated (Dale, 1956) that extracts invariably contain the following acids: citric, malic, succinic, and pyrrolidonecarboxylic. Occasionally oxalic and fumaric acids were found. In addition lactic acid was always found to be present in extracts prepared by the standing procedure. This was probably produced by a fermentation reaction during extraction (Owens, Goodban, and Stark, 1953). No other substances were detected in ether extracts and these results suggested that organic acids might be responsible for the inhibition of salt accumulation. Unpublished results of experiments in which the acids were eluted from chromatograms and tested

for their effect on potassium uptake were unsatisfactory quantitatively, but nevertheless in general agreement with this suggestion.

Experiments with Organic Acids

Dale (1956) established that the titratable acidity of the ether-soluble fraction of extracts indicated a normality of about 0·02 N. This agreed well with the estimated values of concentration made on a spot area basis using paper chromatograms of ether extracts with known concentrations of pure acids as a comparison. In studying the effects of organic acids, therefore, solutions of organic acid salts up to 0·02 N. were used and the pH of the solutions was

TABLE 5

Uptake of Sodium and Potassium (as μeq./g. fresh wt./6 hrs.) by Washed Beet Discs placed in Solutions of Various Salts. Cation Concentration = 0·02 M.

Solutions	Discs washed 1 day		Discs washed 5 days	
	Uptake	Effect	Uptake	Effect
NaCl	13·5±0·40	—	55·2±0·36	—
KCl	13·8±0·27		54·9±0·69	
Na citrate	21·4±0·61	+59%	42·4±0·61	−23%
K citrate	22·0±0·20		41·6±0·52	
Na succinate	21·8±0·35	+62%	43·0±0·49	−22%
K succinate	22·0±0·20		42·2±0·59	

adjusted to 6·0–6·2 using, where necessary, mixtures of acid and the acid salts. In all cases the concentration of cations in the solution was 0·02 M.

It was established that pure solutions of organic acids give results comparable to those obtained using beet extracts. Some typical results are shown in Table 5. Potassium uptake from solutions containing succinate and citrate was greater than from solutions of potassium chloride with discs washed for 1 day, but was smaller with discs washed for 5 days. In other experiments similar effects were obtained with solutions containing malate and fumarate. Uptake from solutions containing acetate, glutamate, oxalate, and lactate was the same as from solutions containing chloride.

Also included in Table 5 are data on the uptake of sodium from sodium chloride, succinate, and citrate. Cooil (1952) found that the stimulating effect of the potassium salts of certain acids on growth of the *Avena* coleoptile was due to potassium ions, since when these were replaced by sodium the effect was lost. Our data show that the effects of organic acids are not associated only with potassium, since sodium uptake is affected to a similar extent (cf. results for manganese uptake).

It is clear that, for discs washed at room temperature, at some stage between 1 and 5 days after cutting, the effect of organic acids on potassium uptake is reversed. To obtain further information on the nature of this reversal two sets of discs were prepared and washed, one at 1° C. and the other at room temperature (20°±2° C). After 2, 4, 7, or 10 days' washing, samples of discs were removed and the uptake of potassium from KCl and potassium succinate solutions in a 6-hour period at 25° C. determined. From the results

shown in Fig. 2 it is clear that for discs washed at room temperature succinate began to have an inhibitory effect on potassium uptake after a washing pretreatment of between 2 and 4 days. With discs washed at 1° C. the onset of the inhibitory phase was retarded, apparently indefinitely. There was, however, an increase in potassium uptake as the washing period was increased,

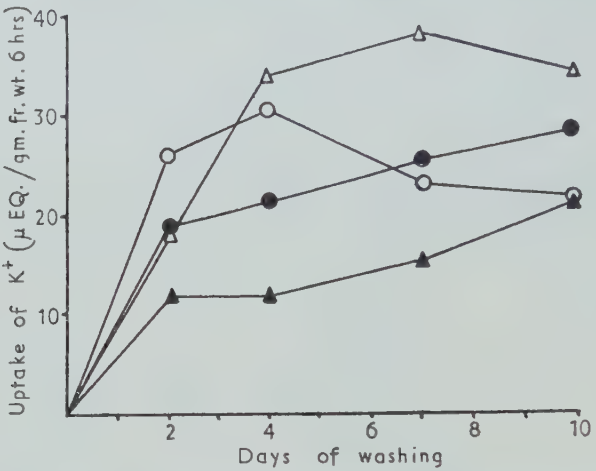


FIG. 2. Uptake of potassium by beet discs in 6 hours at 25° C. from solutions of potassium chloride and potassium succinate after pretreatment at 1° C. or 20° C.

△ In KCl; discs washed at 20° C.
▲ In KCl; discs washed at 1° C.
○ In potassium succinate; discs washed at 20° C.
● In potassium succinate; discs washed at 1° C.

TABLE 6

Oxygen Uptake (μl. O_2/g. fresh wt./hr.) by Beet Discs washed for Various Periods in Water at Room Temperature and then placed in KCl, Potassium Citrate, or Distilled Water

Washing period	1 hour	1 day	2 days	3 days	6 days
Dist. water . . .	104	155	161	154	143
KCl	94	191	193	184	177
K citrate (pH 6·2) . .	88	181	210	207	212

even at this temperature of pretreatment. Similar results were obtained in experiments in which succinate was replaced by malate or citrate, and it was thought possible that with low washing temperatures the slow metabolism of beet discs did not permit an inhibitory effect of the organic anion to develop. The possibility that the organic anions may be metabolized by discs washed at room temperature, causing an effect on respiration, was examined in an experiment in which the respiration of discs in water, KCl and potassium citrate was measured after various lengths of washing pretreatment. The results are given in Table 6.

For discs washed for 1 hour only, respiration was unaffected by the presence

of salts, but in discs washed for 1 day or more the respiration rate in either salt significantly exceeded that in distilled water. Potassium citrate had a larger stimulatory effect than potassium chloride on discs washed for 2 or more days. After the second day of washing respiration showed a decline with all but the citrate treatments. With discs washed for 3 days, the RQ during the citrate

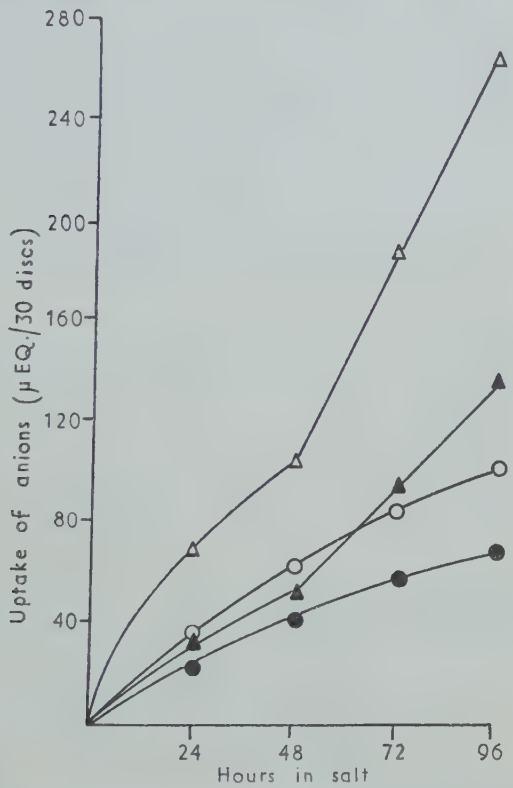


FIG. 3. Uptake of citrate and chloride by beet discs washed for 1 day in water and then placed in various salt solutions for 96 hours at 25° C.

△ Citrate uptake from 0·01 M. dipotassium citrate
▲ Citrate uptake from a mixture of 0·01 M. KCl and 0·005 M. dipotassium citrate
○ Chloride uptake from 0·02 M. KCl
● Chloride uptake from a mixture of 0·01 M. KCl and 0·005 M. dipotassium citrate

treatment reached a value of 1·25, whilst during the other treatments it remained about unity.

It was thought that citrate might be metabolized by a respiratory mechanism and thus disappear from the external medium during an experiment and this was investigated by measuring the uptake of potassium, chloride, and citrate over a 96-hour period, using discs pretreated by washing in water for 1 or 5 days. The experimental solutions consisted of 0·02 M. KCl, 0·01 M. dipotassium citrate (pH 6·2) and a mixture containing 0·01 M. KCl and 0·005 M. dipotassium citrate. The uptake of anions is shown graphically in Figs. 3 and 4.

During the first 24 hours in citrate solutions, discs previously washed in water for 24 hours took up this ion at a rate which exceeded that of chloride from the comparable solution of KCl. After 48 hours, the rate of uptake of citrate by these discs increased to such an extent that the external medium

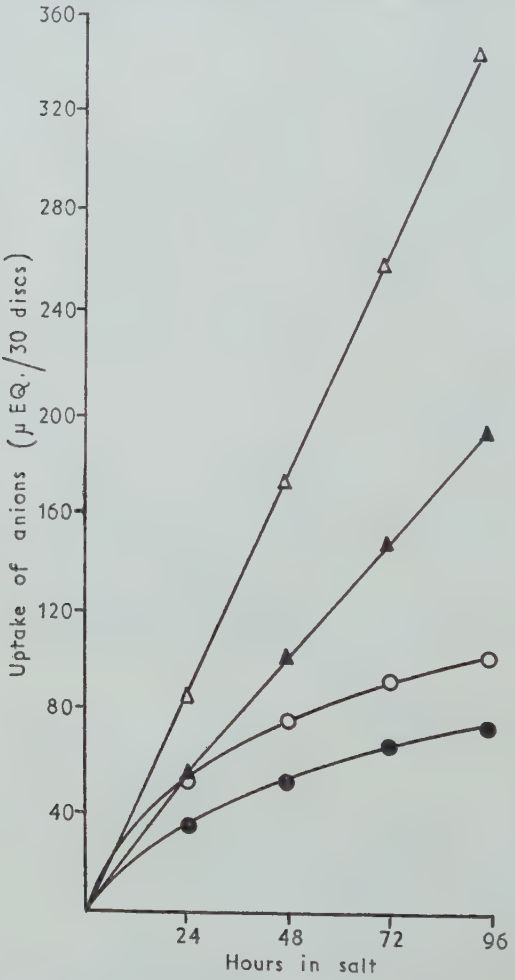


FIG. 4. Uptake of citrate and chloride by beet discs washed for 5 days in water and then placed in various salt solutions for 96 hours at 25° C. Symbols as in Fig. 3.

was completely depleted of the ions in 24 hours. This rate continued until the end of the experiment. With discs washed for 5 days in water, citrate uptake proceeded at maximum rate immediately and continued at this level for 96 hours.

It was uncertain how much of the citrate accumulated by the discs was being metabolized, and it was thought that analysis of the discs from the experiment recorded in Fig. 4 would elucidate this point. An extract was

made from the discs with acid ether and chromatographed in chloroform/ethanol/formic acid. It was found by comparing spot sizes that discs which had been in citrate solution had accumulated small amounts of that acid and rather larger amounts of malic acid. Although it would be unwise to put too much emphasis on these observations since they were unreplicated, evidently only a small part of the citrate absorbed was accumulated.

Turning again to Figs. 3 and 4 it will be noted that discs placed in the mixture of salts took up less chloride than discs in KCl alone. It was uncertain whether this was due to the lower chloride concentration in the mixture or to a competitive effect of the organic anion. An experiment was designed to discover which of the two possibilities was the most likely. Discs were washed for 1 or 4 days and then placed in 0·01 M. KCl, 0·02 M. KCl, or a solution containing 0·01 M. KCl and 0·005 M. dipotassium citrate. After a 6-hour period the chloride uptake was determined (Table 7).

TABLE 7

Uptake of Chloride (μeq./g. fresh wt./6 hrs.) by Beet Discs washed for 1 day or for 4 days and then placed in Solutions of Salts at 25° C.

	Washed for 1 day	Washed for 4 days
0·02 M. KCl	6·2±0·20	22·1±0·40
0.01 M. KCl	3·8	14·3±0·20
0·01 M. KCl+0·005 M. K citrate . . .	3·8	13·6±0·50

The uptake of chloride is clearly dependent upon the concentration of that ion in the medium and is apparently not affected by the concentration of potassium or the presence of citrate. The fact that there was no competition between citrate and chloride suggested that there is a separate system concerned in the uptake of each of the two anions.

Experiments on Manganese Uptake

Having established the effects of beet extract and organic acids on potassium uptake, attempts were made to discover why these results differed from those of Skelding and Rees, by determining the effects of beet extract, and various fractions of it, on manganese uptake.

Solutions of full extract, mother liquor, and ether extract were prepared, concentrated sixfold by gentle heating, and standardized to contain potassium at 0·03 M. and sodium at 0·01 M. 10 ml. aliquots of these solutions were diluted with 50 ml. of 0·001 M. $MnCl_2$ and the uptake of manganese from these and control solutions was determined.

The results of this experiment (Table 8) showed that the inhibition of manganese uptake by the full extract reached 87 per cent., a value considerably in excess of that found for inhibition of potassium uptake. In further contrast to the work on potassium uptake, both ether soluble and insoluble fractions inhibited uptake of manganese, although the full extract always showed a greater effect than either of the fractions.

The course of inhibition by full extract was also studied by using discs washed only for a short time and it was found that the stimulation of cation uptake which was observed when potassium was the test cation did not occur with manganese. In none of the experiments was a stimulation of manganese

TABLE 8

The Uptake of Manganese (μeq./30 Discs/24 hrs.) by Beet Discs washed for 4 days in Water and placed in Solutions containing $MnCl_2$+KCl and $NaCl_2$, and $MnCl_2$ with Various Beet Extracts

Control	Uptake		% inhibition
50 ml. of 0·001 M. $MnCl_2$+10 ml. solution containing 0·03 M. KCl and 0·01 M. NaCl	39·5 35·3 37·5	37·5	—
50 ml. of 0·001 M. $MnCl_2$+10 ml. full extract	7·0 4·0 4·0	5·0	87
50 ml. of 0·001 M. $MnCl_2$+10 ml. mother liquor	13·5 13·5 13·5	13·5	64
50 ml. of 0·001 M. $MnCl_2$+10 ml. ether extract	16·0 18·0 17·5	17·2	53

uptake observed and it seemed important to discover the effects of solutions of organic anions on manganese and concurrent potassium uptake. The experimental solutions used in determining these effects were as follows:

0·001 M. $MnCl_2$	pH 6·1
0·002 M. KCl+ 0·001 M. $MnCl_2$	pH 6·2
0·002 M. KCl	pH 6·2
0·001 M. K_2H citrate 0·001 M. $MnCl_2$	pH 6·2
0·001 M. K_2H citrate	pH 6·1

Discs washed for 1 day only were allowed to take up ions from these solutions for 144 hours and it was found that the presence of manganese reduced the uptake of potassium from both the citrate and the chloride series (Fig. 5). The difference in uptake between the potassium citrate/manganese chloride mixture and the potassium chloride/manganese chloride mixture is statistically significant. The presence of either KCl or potassium citrate also depressed manganese uptake, the effect of citrate being considerably greater than that of chloride (Fig. 6). These discs therefore showed a reduced manganese uptake in the presence of citrate, whilst potassium uptake was stimulated by the presence of citrate. The effect of the organic anions on uptake of cations was again observed with discs washed in water for 4 days, using malate as the test anion (Table 9). However, in this case the uptake of both manganese and potassium was lower in the presence of malate than in the presence of chloride ions.

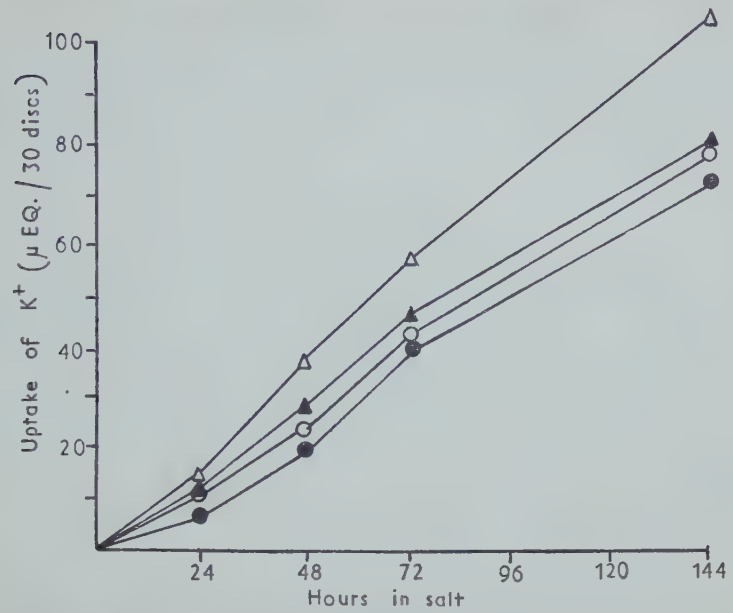


FIG. 5. Uptake of potassium by beet discs, washed for 1 day in water and then placed in solutions containing 0·002 M. KCl (△); 0·002 M. KCl+0·001 M. $MnCl_2$ (▲); 0·001 M. dipotassium citrate (○); and 0·001 M. dipotassium citrate+0·001 M. $MnCl_2$ (●) for 144 hours at 25° C.

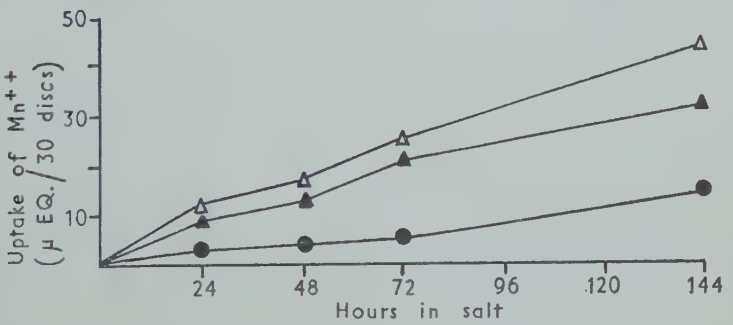


FIG. 6. Uptake of manganese by beet discs washed for 1 day in water and then placed in solutions of 0·001 M. manganese chloride (△); 0·002 M. potassium chloride+0·001 M. manganese chloride (▲); 0·001 M. potassium citrate+0·001 M. manganese chloride (●) for 144 hours at 25° C.

TABLE 9

Uptake of Mn^{++} and K^+ (μeq./30 discs/6 hrs.) by Discs in $MnCl_2$ Solutions with added Potassium Salts

	Mn uptake	K uptake
0·001 M. $MnCl_2$ + 0·002 M. KCl	13·8±0·2	27·7±0·1
0·001 M. $MnCl_2$ + 0·002 M. dipotassium malate	6·0±0·67	16·0±0

The organic anion had a marked inhibitory effect on the uptake of manganese, which was reduced by 56 per cent., whereas potassium uptake was reduced by only 25 per cent. In a control series the uptake of potassium from 0·02 M. KCl exceeded that of manganese from 0·01 M. $MnCl_2$ by about 33 per cent. (expressed as μeq.).

Discussion

Inhibition and the Lag Phase

Results from this study do not support Skelding and Rees's hypothesis that the lag period in beet discs is associated with an inhibitor of salt accumulation, for a number of reasons which are listed below.

1. Beet extracts will only cause inhibition of potassium uptake in discs which are no longer in the lag phase (Table 2). Extracts promote uptake of potassium by discs of both actively growing and dormant tissue which are in the lag phase.

2. Inhibitory extracts can be prepared from well-washed material which is able to accumulate potassium quite readily.

3. The analytical studies reveal that the substances affecting potassium accumulation are organic acids of the citric-acid cycle. Unpublished results of Dale show that in a 4-day washing period the content of citric and malic acid in beet discs decreased by only 10 per cent. There is no evidence, as Skelding and Rees claimed, for a large-scale loss of 'inhibitor' due to either oxidation or leaching processes.

4. From the experiments in which the effects of extracts on manganese uptake were determined, it appears that our extracts were similar in nature to those of Skelding and Rees. However, the mechanism whereby extracts reduce manganese uptake will be shown to be different, at least in part, from that operating in the case of potassium uptake.

5. It has been shown that active extracts can be prepared from young actively growing beet roots as well as from dormant tissue. Discs cut from both types of root show a lag phase in the uptake of potassium, contrary to the views of Skelding and Rees, who claimed that young, growing beet lack the inhibitor which appears as the roots grow older and approach dormancy. There is no evidence from any of our experiments to support the association of an inhibitor with the lag phase in beet. Skelding (1956, 1957) found that high concentrations of CO_2 inhibited uptake of manganese by beet discs. This is not surprising, but before linking CO_2 inhibition with a hypothetical inhibitor it would be wisest to establish the reality of the latter, which has not yet been done.

The Mechanism of the Organic Acid Effect

The results show clearly that certain organic acid anions which occur in beet extracts have a marked effect on both respiration and salt accumulation. From the respiration data it is apparent that a stimulation of respiration in

discs washed for more than 2 days in water is caused by citrate; other data (Dale, 1956) show that malate and succinate have similar effects. The stimulation of oxygen uptake in washed material is accompanied by an increased RQ. This result is in agreement with those of Bennet-Clark and Bexon (1943) and Turner and Hanly (1949). These workers found that the effects of organic acids on respiration were greatest at pH values 4·0–5·5. At higher pH values (*circa* 7·0) Turner and Hanly found that the stimulation of oxygen uptake was much less marked, whilst the RQ was usually less than 1·0. Ulrich (1941, 1942), studying the uptake of ions by excised barley roots, showed that when cations were taken up in excess of anions, organic acid anions tended to accumulate in the tissues, whereas when anions were taken up in excess of cations, organic acids were broken down with a concomitant RQ in excess of 1·0. Our results fit in well with these findings, since discs which take up markedly more citrate than potassium show an increased RQ (i.e. from 1·0 to about 1·3). Both Turner and Hanly, and Bennet-Clark and Bexon found that at low pH values the RQ of discs in organic acids exceeded by a considerable amount that theoretically possible for complete oxidation of the substrate in question. They concluded that, as well as the complete oxidation of the organic-acid substrate, a second reaction involving partial oxidation occurred. At the pH values used in our experiments the high RQ values were not observed, nevertheless the possibility of two types of oxidation reaction occurring must be borne in mind.

The very large uptake of citrate observed in these experiments poses two problems. The first of these concerns the fate of the absorbed citrate. Probably some, and possibly all, of this substance is respired. At the same time the rather dubious evidence for the accumulation of malate in discs left in citrate for 96 hours may mean that a partial conversion of citric acid occurs. Jacobson and Ordin (1954) showed that changes in the malic-acid fraction could account entirely for the 'Ulrich effect' in barley roots.

The second problem posed by the large uptake of citrate concerns ionic balance in the external medium. It seems that the greater uptake of anions over cations can only be explained by an ion exchange phenomenon, or by uptake of organic anions in association with hydrogen ions. There is no information on the mechanism of this reaction, or on the nature of the exchanging anion, if exchange occurs. Nor is there any evidence concerning the uptake of anions from solutions of malate or succinate, though the behaviour of discs in such organic-acid solutions resembles that of discs in citrate.

The action of citrate on potassium uptake can be explained tentatively as follows: In freshly cut discs it is apparent from the results in Table 7 and Fig. 3 that chloride is not taken up to nearly the same extent as is citrate. If absorption of cations is limited by the rate of absorption of anions, the more rapid absorption of potassium from solutions of citrate than from solutions of chloride is understandable. As the discs age during the washing procedure, both the citrate and the chloride accumulatory mechanisms develop but the

uptake of citrate apparently becomes divorced from the potassium-accumulating mechanism and linked with other metabolic processes. Hence with discs washed for 4 days, uptake of potassium is greater from chloride because the potassium/chloride accumulatory system is by then more effective than that concerned in the uptake of potassium in association with citrate.

It seems clear that discs of beet have a propensity for potassium accumulation from the moment they are cut, since discs washed for only a short time will take up large quantities of potassium from the solutions of potassium salts of the organic acids. It would seem that the lag phase in potassium uptake is an artefact and merely a reflection of a time lag in the development of a chloride accumulatory system. It may be that the development of a chloride accumulating system is in itself a response to the artificial conditions following the cutting of the discs and their washing in water.

Since this work has shown the importance of anions in the accumulatory mechanism it is of interest to consider the results in relation to the theory of anion respiration due to Lundegårdh (1954). In this paper Lundegårdh explains the low value of the ratio:

mols. absorbed anions/mols. oxygen consumed in anion respiration

which might have been expected to reach a value of 4 on the basis of his theory (Robertson, 1941) as being due to the effects of 'native anions' circulating in the plant tissues. These 'native anions' are held to consist in part of organic acids produced in the cytoplasm, and Lundegårdh suggests that these large organic anions compete with small inorganic anions from the medium for the cytochrome system. He holds that the latter will be favoured because of their greater mobility. From our results there is evidence that citrate and chloride do not compete for accumulation. Chloride uptake is shown to be dependent upon the concentration of chloride in the medium irrespective of the presence or absence of citrate, whilst citrate uptake from a 1:1 mixture exceeds that of chloride. On the Lundegårdh hypothesis it would be expected that some measure of competition would occur between the two anions.

Effects on the Uptake of Manganese

The results of the experiments with manganese can conveniently be summarized under four headings.

1. Beet extracts and solutions containing organic anions caused a large inhibition of manganese uptake which was greater than that found for potassium uptake by discs from extracts of similar concentration.

2. Both ether-soluble and -insoluble fractions showed an effect in inhibiting manganese uptake, although each separately showed a smaller effect than the full extract. In contrast, the inhibition of potassium uptake has been shown to be a property of the ether-soluble fraction only.

3. No stimulating effects of extracts or organic anions on manganese uptake by discs washed for short periods were observed.

4. Inhibition of uptake was never complete. Even with the full beet

extract the maximum inhibition obtained was 87 per cent., whereas Skelding and Rees (1952) reported complete inhibition of manganese uptake.

The explanations advanced to account for the effects of extracts and organic anions on potassium uptake are not entirely adequate to explain the findings with manganese. The following is a tentative explanation which is not opposed by any of the experimental facts.

The importance of manganese as a cofactor in certain enzymic reactions is well known and has led a number of workers to study the properties of chelate compounds of manganese and certain organic molecules. Li, Westfall, Lindenbaum, White, and Schubert (1957) have reported the manganese/citrate complex to have a log K_f value of 3·54 indicating a relatively stable structure. Martell and Calvin (1952) suggest that the association between manganese and amino-acids and peptides is even stronger than that with citrate since amino, phenolic, and in some cases imidazole groups can act as ligands as well as hydroxyl and carboxyl groups.

Aqueous extracts of beet root contain ether-soluble organic acids some of which are known to possess chelating properties. They also contain amino-acids which can complex with ions such as manganese. It is suggested that the inhibition of manganese uptake is due to the complexing of this ion by compounds in the aqueous extract. Such a binding would reduce the amount of manganese ion available for uptake by beet discs.

The following equations summarize the hypothesis.

(*a*) *Discs in $MnCl_2$ alone*

Mn^{++} + binding sites on beet cells $\rightleftharpoons$ (Mn site complex).

The nature of the binding site is immaterial to the argument.

(*b*) *Extract in presence of $MnCl_2$*

Mn^{++} + extract ligands $\rightleftharpoons$ (Mn extract) complex.

It is suggested that in Skelding and Rees's experiments the manganese in their media was predominantly bound to the extract ligands.

In our experiments, however, the extracts were less concentrated and hence less manganese would be bound and more available for uptake. It should be noted that Skelding and Rees found that extracts half as concentrated as ours had only a slight inhibitory effect.

In the experiments with citrate and malate, incomplete inhibition may be due to either a low stability or association constant for the binding reaction, or to the use of low concentrations of the organic anion. Either of these factors would result in a high manganese ions/manganese complex ratio and enable a measurable uptake of manganese to occur.

If complexing of manganese is the explanation of these results, it follows that both the physical and metabolic phases of uptake are affected indirectly, since the effect of the extract is to render manganese unavailable for uptake.

Thus the divergences between our results and those of Skelding and Rees can be considered to be at least partially resolved. These authors were

unfortunate in choosing as their test cation one whose chemistry is rather complicated, and were unwise to argue for a general salt-accumulation inhibitor on the basis of results for manganese alone.

Acknowledgements

It is a pleasure to acknowledge the continual interest and encouragement given to us in this work by Professor T. A. Bennet-Clark, F.R.S.

One of us (J. E. D.) also acknowledges the receipt of an A.R.C. grant.

Literature Cited

Asprey, G. F., 1933: Studies in Antagonism II. The Effect of Previous Immersion of Potato Tuber Tissue in Solutions of Monovalent, Divalent, and Trivalent Cations on the Subsequent Absorption of the Ammonium Ion. Proc. Roy. Soc. B, **113**, 71–82.

Bennet-Clark, T. A., and Bexon, D., 1943: Water Relations of Plant Cells. III. The Respiration of Plasmolysed Tissues. New Phyt., **42**, 65–92.

Bentley, L., 1953: Ph.D. Thesis, University of London.

Cooil, B. J., 1952: Relationships of Certain Nutrients, Metabolites and Inhibitors to Growth in the Avena Coleptile. Plant Physiol., **27**, 49–69.

Dale, J. E., 1956: Ph.D. Thesis, University of London.

—— and Sutcliffe, J. F., 1956: Inhibition of Salt Absorption in Red Beet Root Tissue. Nature, **177**, 192–3.

Furman, N., and Low, G. W., 1935: The Use of Concentration Cell in Quantitative Analysis. 1. Estimation of Small Amounts of Chloride in Salts. J. Amer. Chem. Soc., **57**, 1585.

Isherwood, F. A., and Hanes, C. S., 1953: Separation and Estimation of Organic Acids on Paper Chromatograms. Biochem. J., **55**, 824–30.

Jacobson, L., and Ordin, L., 1954: Organic Acid Metabolism and Ion Absorption in Roots. Plant Physiol., **29**, 70–75.

Li, N. C., Westfall, W. M., Lindenbaum, A., White, J. M., and Schubert, J., 1957: Manganese-54, Uranium-233 and Cobalt-60. Complexes of some Organic Acids. J. Amer. Chem. Soc., **79**, 5864.

Lundegårdh, H., 1954: Anion Respiration. Symp. Soc. Exp. Biol. **8**, 262–96.

McArdle, B., 1955: A Modified Method for the Micro Determination of Citric Acid. Biochem. J., **60**, 647–50.

Martell, A. E., and Calvin, M., 1952: The Chemistry of the Metal Chelate Compounds. New York.

Middleton, L. J. 1954: Ph.D. Thesis, University of London.

—— 1956: Salt Respiration in Storage Tissue. New Phytol., **55**, 117–19.

Owens, H. S., Goodban, A. E., and Stark, J. S., 1953: Fractionation of Organic Acids in Sugar Beet by Ion Exchange Resins. Anal. Chem., **25**, 1507–9.

Rees, W. J., 1949: The Salt Relations of Plant Tissues IV. Some Observations on the Effect of the Preparation of Storage Tissues on its Subsequent Absorption of Manganese and Chloride. Ann. Bot., N.S., **13**, 29–51.

—— and Skelding, A. D., 1950: An Inhibitor of Salt Absorption in Root Tissue of Red Beet. Nature, **166**, 823–4.

Robertson, R. N., 1941: Studies in the Metabolism of Plant Cells I. Accumulation of Chloride by Plant Cells and its Relation to Respiration. Aust. J. Exp. Biol. Med. Sci., **19**, 265–78.

Skelding, A. D., and Rees, W. J., 1952: An Inhibitor of Salt Absorption in the Root Tissues of Red Beet. Ann. Bot., N.S., **16**, 513–29.

—— 1956: Effect of Carbon Dioxide on the Manganese Absorption of Red Beet Tissue. Nature, **177**, 1038.

—— 1957: The Effect of Carbon Dioxide on the Absorption of Manganese by Root Tissues of Red Beet. Ann. Bot., N.S., **21**, 121–42.

Stark, J. S., Goodban, A. E., and Owens, H. S., 1951: Paper Chromatography of Organic Acids. Anal. Chem., **23**, 413–15.

Stiles, W., and Dent, K. W., 1946: The Salt Relations of Plant Tissues. III. Further Observations on the Absorption of Manganese Chloride by Storage Tissue. Ann. Bot., N.S., **10**, 203–22.

Sutcliffe, J. F., 1952: The Influence of Internal Ion Concentration on Potassium Accumulation and Salt Respiration of Red Beet Root Tissue. J. Expt. Bot., **3**, 59–76.

—— 1954: Cation Absorption by Non-growing Plant Cells. Symp. Soc. Exp. Biol., **8**, 325–42.

Turner, J. S., and Hanly, V. F., 1949: Succinate and Plant Respiration. New Phyt., **48**, 149–71.

Ulrich, A., 1941: Metabolism of Non-volatile Organic Acids in Excised Barley Roots as Related to the Cation–Anion Balance during Salt Accumulation. Amer. J. Bot., **28**, 526–37.

—— 1942: Metabolism of Organic Acids in Excised Barley Roots as Influenced by Temperature, Oxygen Tension, and Salt Concentration. Ibid., **29**, 220–7.

Vogel, A. I., 1951: Quantitative Inorganic Analysis. Longmans, London, p. 704.

A Population Study of *Spergula arvensis*

II. Genetics and Breeding Behaviour

BY

JUNE K. NEW[1]

(*University College, London*)

Abstract

Genetical work has shown that one locus and two alleles are involved in the inheritance of a seedcoat character of *Spergula arvensis* and that the heterozygous plants are intermediate between the two types of homozygous plants. It is thus possible to translate directly the observed geographical distribution of plant frequencies into distribution of gene frequencies. The inheritance of a hairiness character is less straightforward, but in this case also there is a close relation between phenotype frequency distribution and gene frequency distribution.

Using the fact that plants heterozygous for the seedcoat character are easily recognizable, it is possible to make an estimate of the amount of outbreeding in nature. The values obtained range from 0 to 3 per cent.

Introduction

IT was shown in an earlier paper (New, 1958) that there are differences in the distribution of two seedcoat forms (the presence or absence of papillae) and two hairiness forms (medium or densely hairy) of *Spergula arvensis*. There are clines in the distribution of both these pairs of characters, the proportions of the non-papillate and the densely hairy forms increase with increasing distance north-north-west across the British Isles. This was established from a study of the phenotypes by sampling *S. arvensis* populations in the field. It is therefore of interest to know what relation the distributions of the phenotypes have to the distributions of the genotypes. Because the plants belonging to the two classes of seedcoat and the two classes of hairiness are found growing side by side in many localities, the genetic control of the

[1] Now c/o Zoology Dept., University College, Mona, St. Andrew, Jamaica, B.W.I.

[Annals of Botany, N.S. Vol. 23, No. 89, 1959.]

characters is obviously not markedly affected by the environment. There remains, however, the possibility that dominance is obscuring heterozygosity and causing an overestimate of the dominant class. A very few plants bearing seeds with a smaller number of papillae were found and recorded (number of papillae about 60, instead of the usual 120 per seed). It seemed possible that these were heterozygous. The results of artificial crosses described in this paper confirm this and the inheritance proves to be controlled by a single gene difference. Experimental crosses were also made to study the inheritance of the hairiness character in order to see whether either form is dominant.

It was mentioned above that both the seedcoat and hairiness characters show clines in a north-north-west direction across the British Isles. The evidence from field observations suggested that this is not a linkage or pleiotropic phenomenon. Genetical data also relevant to this question is given in this paper.

Several authors have pointed out that where two forms exist side by side in the same habitat there is a *prima facie* case for hybrid vigour. The evidence for vigour has therefore been investigated.

For the interpretation of population structure it is most important to know the breeding behaviour of the species. This has rarely been studied in wild species (the exception is some work on grasses) although many workers (Bateman, 1947; Hayes and Garber, 1927, &c.) have determined the amount of crossing between pure lines of crop plants. Wild species are usually classed simply as mainly selfed or mainly crossed, self-compatible or self-incompatible. Because the inheritance of the seedcoat character in *S. arvensis* is so simple it is possible, with certain assumptions, to estimate the proportion of crossing in nature from the observed proportion of heterozygous plants. The most important prerequisite is to be able to classify the seeds accurately as homozygous or heterozygous. The plasticity of the homozygous and heterozygous plants for the seedcoat character was therefore studied to see whether the three types could always be distinguished when growing together. The results show that it can be assumed that it is possible to distinguish them under all conditions likely to be encountered in the British Isles. On this assumption an estimate of the amount of crossing has been made for several localities.

The Inheritance of the Seedcoat and Hairiness Characters

Materials and methods: Plants grown from seeds collected in various localities in the British Isles and Norway were used for the crosses.

The technique I used was to dissect out the anthers from the lower buds with a Borrodaile needle, using a magnification of $\times 10$ under a dissecting microscope. The flowers are homogamous and buds were selected at the stage when it was just possible to remove the anthers without causing them to dehisce. This was when the buds were about 2 mm. long and before the petals had grown longer than the sepals—about 2–3 days before the flowers first opened. The flowers were pollinated immediately by placing a mature anther on the stigmatic surface with a needle. The number of anthers in each

flower ranges from 4 to 10 and the number varies from flower to flower on the same plant. This means that there is no way of checking that all the anthers have been removed except by inspection. Flowers which had been emasculated but not artificially pollinated were left to develop as controls. The treated flowers were marked by tying a piece of coloured cotton round the peduncle. The whole plant was then covered with a bag to prevent insects from visiting the flowers.

About 2 weeks after pollination the seeds are mature and ready for collection. None of the control flowers which had been left unpollinated produced seed and the hand-pollinated flowers produced an average of 10 seeds. Naturally pollinated flowers usually produce 15–20 seeds. It is possible that the reduction was due to damage of the ovary during dissection.

Each seed is divided in half by a circular wing. It was established that the number of papillae on each half is similar and thereafter only one side was counted. All the counts have been multiplied by two in the description of the results, as an estimate of the number of papillae on a seed. Hairiness was measured by counting the number of hairs per $1\frac{1}{2}$ cm. as seen in silhouette on the lowest internode. Densely hairy plants have about 40 hairs per $1\frac{1}{2}$ cm. and medium hairy have about 5 hairs per $1\frac{1}{2}$ cm.

Results for seedcoat: The hybrids produced from crossing a non-papillate-seeded plant with a papillate-seeded plant have seeds with an intermediate number of papillae. The number may vary on any one plant from 0 to about 80. The number of papillae on every seed of the papillate parent is normally greater than 120. The number of papillae is somewhat affected by the environment, and I shall consider this point in more detail later, but in the following description of the results of the crosses I have classified as intermediates the plants with a variable number of papillae (less than 80) on the seeds. The plants classified as non-papillate have no papillae on any of the seeds.

The results show that there is a segregation of non-papillate, intermediate, and papillate plants in the F_2 generation which is consistent with a theoretical ratio of 1:2:1. The offspring of reciprocal crosses are alike and backcrosses give a ratio of intermediate to papillate or non-papillate of 1:1. This must mean that there are two alleles at one locus involved in the control of papillae on the seedcoat. The results of the crosses are given in Table 1. Several crosses were carried out using material from different sources in order to determine whether the hybrids were intermediate and distinguishable from the parents in all cases. All the crosses gave recognizably intermediate hybrids when grown in the fluctuating conditions of the greenhouse.

The variability of the hybrid: The variation from seed to seed in the number of papillae on one hybrid plant is somewhat unexpected since the seedcoat has the genetical constitution of the mother plant and is therefore the same for all seeds of one plant. It seemed possible that the genetic constitution of the embryo affects the expression of the genes of the seedcoat. To test this, the F_1 seeds from a single cross were sorted into two classes according to the number of papillae on the seed: 0–40 and 41–80. Segregation in the F_2 generation

gave the same ratio for each class and therefore proved that there is no genetical difference between these two classes. The results are given in Table 2.

TABLE 1

Cross	F_2 segregation			χ^2 on 1:2:1 hypoth.	Approx. p
	Non-pap.	Intermed.	Pap.		
Non-pap. Norway × pap. Leicestershire	24	44	25	0·17	0·90
Backcross to non-pap. Norway	15	10	0	—	—
,, to pap. Leicestershire	0	0	1	—	—
♂ Non-pap. Norway × ♀ pap. Leicestershire (*reciprocal*)	6	7	6	1·32	0·50
Backcross to non-pap. Norway	23	27	3*	—	—
,, to pap. Leicestershire	0	4	6	—	—
Non-pap. Northern Ireland × pap. N.I.	3	12	7	1·64	0·45
Backcross to non-pap. N.I.	3	8	0	—	—
,, to pap. N.I.	0	2	2	—	—
Non-pap. Essex × pap. Essex	9	10	4	2·61	0·30
Non-pap. Leicestershire (*a*) × pap. Essex (*a*)	8	18	6	0·75	0·70
,, ,, (*b*) × ,, ,, (*b*)	7	13	4	0·92	0·40
,, ,, (*c*) × ,, ,, (*c*)	10	15	10	0·72	0·70
Non-pap. Norway × pap. Essex	16	19	12	2·41	0·30
Non-pap. Caithness × pap. Essex	3	12	4	1·42	0·50
Non-pap. Northern Ireland × pap. Essex	15	22	10	1·26	0·55
Non-pap. Caithness × pap. Northern Ireland	10	16	5	1·65	0·45

* Probably a labelling accident.

TABLE 2

Segregation from Two Classes of Seed from One Hybrid Plant

Parent seed class	F_2 segregation			χ^2 on 1:2:1 hypoth.	Approx. p
	Non-pap.	Intermed.	Pap.		
0–40	15	33	20	0·80	0·75
41–80	9	11	5	1·64	0·40

Results for hairiness: These results are difficult to interpret because of the effect of environment on this character. Plants grown in autumn and winter under conditions of low light intensity and temperature tend to be less hairy. However, seeds from selfed parents were grown alongside both the F_1 and F_2 generations and so comparisons were possible. In each of 5 crosses between densely and medium hairy plants, the F_1 plants were intermediate in hairiness. Plants of the F_2 generation showed a considerable range of hairiness but there were no obvious segregation ratios. Estimates of the number of genes involved varied very much with the different crosses (from 20 to 1). For a more detailed description of the results see New, 1957.

Association of hairiness and seedcoat characters: It was mentioned before that dense hairiness and non-papillate seedcoats share a similar geographical distribution and the results given here are concerned with deciding whether this is a linkage or pleiotropic phenomenon, or neither. Five different crosses of non-papillate densely hairy with papillate medium hairy were made and the hairiness and seedcoat class of each of the F_2 plants was determined. The results are given in Table 3 and it will be seen that there is no association between non-papillate seedcoats and dense hairiness or between papillate seedcoats and medium hairiness. The conclusion is that the similarities of distribution are not due to linkage or to a pleiotropic effect.

TABLE 3

The Classification of F_2 Plants according to both Hairness and Seedcoat

Seedcoat class	Hairs per 1½ cm.* 0	0·1–15	15·1–30	⩾ 30·1	Total
Non-papillate	23	46	13	7	89
Intermediate	41	70	11	13	135
Papillate	22	26	10	4	62
Total	86	142	34	24	286

* Average for all branches on each plant.

$\chi^2 = 5{\cdot}702$
d.f. = 6
$p \simeq 0{\cdot}5$

Hybrid vigour: There is a small amount of evidence from the relative rates of germination of selfed and hybrid seeds which favours a theory of hybrid vigour. An increased rate of germination would seem to be of possible selective advantage since *S. arvensis* continues to produce seeds as long as the environmental conditions are favourable for growth. Other things being equal, an earlier germination would increase the length of the growing season and therefore the number of seeds produced.

I noticed in some of the early crossing experiments that seeds containing hybrid embryos germinated more readily than seeds with embryos formed by selfing (of course, in both cases the seedcoats were homozygous). This observation was later investigated in more detail by two series of crosses. In one series 7 plants originating from 2 widely separated localities (Cumberland and Essex) were crossed in most of the possible combinations (17 out of 21) and the F_1 seeds and seeds from the selfed parents were put to germinate under identical conditions in a greenhouse. In 4 cases reciprocal crosses were made to see what effect the seedcoat had on germination. Out of the 17 crosses, after 8 days' germination 3 showed a significantly ($p < 0{\cdot}01$) higher germination proportion than either of their selfed parents. In each of these 3 cases, after a further period the germination proportion of one of the selfed parents rose to be as high as that of the hybrid—hence the difference is only in rate and not in final germination proportion. Four crosses showed an insignificantly higher germination proportion, in 9 crosses the germination proportion was intermediate between that of the two selfed parents and in 1 cross was slightly below (insignif.) that of the inferior parent. There was some difference between reciprocal crosses but in neither case was it sufficiently great to alter the relation to the selfed parents.

In the second, smaller, series of crosses 4 plants from 3 different localities (Northern Ireland, Essex, and Caithness) were crossed in all combinations. There was a significant ($p < 0{\cdot}001$) increase in germination rate of F_1 over both selfed parents in 1 case only. There was an insignificant increase in 3 cases, one cross was intermediate and in one there was an insignificant decrease.

The presence or absence of vigour could not be correlated with the degree of geographical isolation of the parental material or with genetical differences in obvious characters such as seedcoat and hairiness. These results are similar to those of Hagberg (1953) from selfed Galiopsis species where he finds vigour, the degree of which is not associated with the degree of geographical or ecological differentiation of the parents. The existence of hybrid vigour in selfed species is contrary to the predictions of some authors and is not consistant with Dobzhansky's (1955) hypothesis suggesting that alleles or gene complexes which have become mutually adjusted by natural selection give rise to hybrid vigour.

There was no evidence of hybrid vigour as measured by the dry weights of shoots produced.

The Effect of the Environment on the Number of Papillae on Hybrid Seedcoats

As I pointed out in the section on the inheritance of the seedcoat character, the number of papillae on hybrid seeds is variable. The number may vary from 0 to about 80 on the seeds from a single plant. It was shown that this is not due to the effect of the genotype of the embryo and it must therefore be an environmental effect, either internal (e.g. the age of the plant) or external. It was also noted that the number of papillae varied more between capsules than within one capsule. For example, on one plant the following were the counts for five randomly selected capsules:

8, 14, 12, 4, 18.
16, 10, 34, 42, 60.
0, 8, 2, 18, 0.
8, 22, 42, 14, 12.
80, 70, 80, 74.

To investigate the effect of the external environment and age on the number of papillae, hybrid plants were grown at the following temperatures: (*a*) 21° C. (constant), (*b*) 18° C. (constant), (*c*) outside in June, July, and August, (*d*) in a greenhouse during the same period (hotter than outside during the day). Samples of seed were taken when the plants were 12 and 17 weeks old and the number of papillae on each seed was counted. Four different crosses were used and the results are given in Table 4. It will be seen that there is a marked effect of temperature on the number of papillae. As the temperature increases, the number of papillae per hybrid seed decreases. There is no marked trend with the age of the plants.

Under all conditions except 21° C., seeds homozygous for papillate seedcoats producced more than 120 papillae each. At 21° C. an average of 100 papillae were produced. Under no conditions did seeds homozygous for non-papillate seedcoats produce papillae.

It is therefore clear that it is possible to distinguish between homozygotes and heterozygotes grown under the same conditions and it can safely be

TABLE 4

Table showing the Effect of the Age of the Plant and the Environmental Conditions on the Number of Papillae on Hybrid Seeds

	Age of plants		Green-house	Garden	21° C.	18° C.
Cross A	12 wks.	Av. no. pap. per seed	23·2	40·8	3·0	15·6
		Range	10–50	10–90	0–10	0–70
		No. seeds examined	9	41	13	33
	17 wks.	Av. no. pap. per seed	21·4	40·8	7·4	30·0
		Range	0–50	30–90	0–10	
		No. seeds examined	14	45	3	1
Cross B	12 wks.	Av. no. pap. per seed	16·6	65·2	6·6	23·0
		Range	0–90	30–90	0–10	0–70
		No. seeds examined	19	49	26	17
	17 wks.	Av. no. pap. per seed	56·6	71·0	12·2	20·0
		Range	10–90	30–90	0–20	10–30
		No. seeds examined	33	47	18	2
Cross C	12 wks.	Av. no. pap. per seed	58·0	75·0	1·4	25·6
		Range	50–70	30–90	0–10	10–50
		No. seeds examined	5	48	27	31
	17 wks.	Av. no. pap. per seed	33·2	76·8	12·4	10·0
		Range	0–90	30–90	0–20	0–30
		No. seeds examined	56	48	29	5
Cross D	12 wks.	Av. no. pap. per seed	21·2	62·8	3·0	14·6
		Range	0–90	10–90	0–10	0–50
		No. seeds examined	27	47	55	30
	17 wks.	Av. no. pap. per seed	32·8	95·2	0	14·2
		Range	0–50	30–100		0–50
		No. seeds examined	80	46	35	54

assumed that all the heterozygous plants encountered in the field were identified as such. Possibly in a climate hotter than that of the British Isles the heterozygous plants would be indistinguishable from non-papillate ones.

THE AMOUNT OF SELFING AND CROSSING IN NATURE

The small number of plants found in nature which were heterozygous for the seedcoat character must have been produced by crossing and not by

mutation for the following two reasons: (*a*) none were found where the plants were almost completely or completely of one form—mutation rate would not depend on the proportions of the two forms; (*b*) estimates of the mutation rate in higher plants usually suggest a rate of 2–4 per million, whereas in this case there were 20 plants heterozygous for the seedcoat character in the 4,000 I examined.

From observations of the proportion of heterozygous plants in a population it is possible to estimate the proportion of crossing per generation which this represents. Three assumptions are necessary:

1. That the proportion of crossing is constant from generation to generation.
2. That the heterozygous plants have no selective advantage or disadvantage compared with the homozygous plants.
3. That crossing, when it does occur, is at random in respect to seedcoat.

Validity of these assumptions:

1. Observations were made in two consecutive years in Cheshire and the proportions of heterozygous plants differed only slightly (0·016 in 1955 and 0·024 in 1956). These two years differed extremely in climate.
2. As mentioned before, there is some evidence of hybrid vigour and this would tend to cause an overestimate.
3. There is evidence to support this. Experimental crosses showed that there are no differences between the number of seeds set per capsule in like crosses (pap. × pap. and non-pap. × non-pap.) and the number set in unlike crosses (pap. × non-pap.). Neither are there any differences between like and unlike crosses in the proportion of capsules which set seed. The figures are given in Table 5.

TABLE 5

	Like crosses	Unlike crosses
Av. number of seeds per capsule . .	7·3	6·2
Variance	4·616	4·683
Number of crosses	12	10
$t = 1{\cdot}124$		
$p = 0{\cdot}30$		
Av. proportion of fertile capsules . .	0·806	0·746
Variance	0·056	0·045
Number of crosses	12	10
$t = 0{\cdot}619$		
$p = 0{\cdot}50$		

No differences such as time of flower opening, date that flowers are produced, &c., have been observed associated with the seedcoat character which might prevent crossing.

Estimate: Let H be the observed proportion of plants heterozygous for the

seedcoat character. This is made up of first generation hybrids and segregates from crosses in all preceding generations.

Let x be the proportion of 1st (nth.) generation hybrids.

then $\frac{1}{2}x$ will be the proportion of heterozygous plants from the preceding $(n-1)$ generation, because the inheritance is simple Mendelian.

$\frac{1}{4}x$ will be the proportion from generation $(n-2)$

$\frac{1}{8}x$ " " " " $(n-3)$

&c.

$H = x+\frac{1}{2}x+\frac{1}{4}x+\frac{1}{8}x \ldots$

$= 2x$

$x = H/2$

Let a be the observed prop. of P genes in the population

Let $b\ (= 1-a)$ " " N " " "

Let k be the proportion of unlike cross fertilizations per generation, i.e.

$$\frac{\text{actual } PN}{\text{maximum possible } PN}$$

From the Hardy-Weinberg Law the maximum possible PN is $2ab$ $\therefore$ $k = x/2ab$

Substituting observed values of H, a, and b the values of k are obtained. The results are given in Table 6.

TABLE 6

Locality	Number of plants			k
	Pap.	Intermed.	Non-pap.	Prop. crosses
Cheshire '55 . .	127	9	416	0·022
" '56 . .	54	5	149	0·030
Derbyshire . . .	107	2	96	0·010
Herefordshire . .	39	0	150	0
Merionethshire . .	65	0	178	0
Perthshire . . .	51	1	167	0·008
Shropshire . . .	58	3	372	0·014
Surrey . . .	225	0	382	0
Westmorland . .	40	1	179	0·008
Wigtownshire . .	31	3	186	0·027

Because it can be assumed that the crosses $P\times P$ and $N\times N$ take as easily as the cross $P\times N$, k represents the total actual crosses over the total possible crosses, i.e.

$$k = \frac{\text{Actual } (a^2+2ab+b^2)}{\text{Possible } (a^2+2ab+b^2)}$$

The results show that there is a small amount of crossing in *Spergula arvensis*, varying between 0 and 3 per cent.

Certain Syrphids (Hoverflies) appear to be particularly attracted to *S. arvensis* but other insects are very occasionally seen working the flowers.

Discussion

It is clear from the experiments to elucidate the inheritance of the seedcoat character that it is a simple case of two alleles, one locus, and that there is no dominance. It may therefore be concluded that the cline found in this character is a cline for gene frequency as well as for phenotype frequency. The inheritance of the hairiness is less straightforward but since there is no evidence of dominance, the cline of the hairiness phenotypes must be closely related to a cline in the genes determining this character.

There is some evidence of hybrid vigour as measured by the relative rates of germination of homozygous and heterozygous seed. Because there is such a low percentage of crossing and so few hybrids are ever produced, the vigour is unlikely to play a large part, in the case of *S. arvensis*, in explaining how two forms continue to grow side by side in some localities when, as Fisher has shown, only a very slight selective advantage of one form would be expected to completely suppress the other form in time.

It is interesting that in this predominantly self-fertilizing species there is a certain amount of outbreeding. The opinion has been expressed that many supposedly completely selfed species show a small amount of outbreeding and the results for *S. arvensis* support this. Several authors have suggested that selfing gives immediate fitness through the certainty of reproduction rather than flexibility, through recombinations, but that it is of short-term rather than long-term evolutionary value. However, Stebbins (1950) has pointed out that annual habit and a high reproductive rate means that the rate of evolution of a species with only a small amount of outbreeding may be as great as a perennial with a lower reproductive rate and a higher percentage recombination. Thus, mainly selfed species may not have such a poor evolutionary outlook as is sometimes suggested. This idea is supported by the results of work on the supposedly selfed *Camelina sativa* (Zinger, 1909). *C. sativa* shows good evidence of a rapid rate of evolution and adaptation to life as a weed of linseed crops and this is difficult to account for if it is completely selfed. A careful reinvestigation of the breeding behaviour of this species would be of interest. Where the inheritance of a character is simple Mendelian, the method described in this paper is a very convenient one for estimating breeding behaviour.

Because there is so little crossing in *S. arvensis*, the role of pollen in the long-distance transfer of genes must be small and cannot play other than a very small part in maintaining the dynamic equilibrium of the cline. The problems of the maintenance of the cline and the existence of two forms side by side in some localities are discussed at greater length in the previous paper (New, 1958) and it is suggested that continual long-distance dispersal of *S. arvensis* seeds and possibly also fluctuating selective forces provide the explanations.

Summary

1. Genetical experiments were carried out in order to find what relation the clines in the phenotype frequencies of seedcoat and hairiness characters,

known to exist across the British Isles, have to the distribution of the genotypes.

2. The inheritance of the seedcoat character proves to be determined by a single gene difference and there is no dominance. It may therefore be concluded that the cline in phenotype frequencies is exactly paralleled by a cline in gene frequencies.

3. The variability in the number of papillae on the hybrid seedcoat is due to environmental effects and not to the genetic constitution of the embryo.

4. In most cases there is no evidence of dominance in the crosses between medium-hairy and densely hairy plants and it may therefore be concluded that the cline in phenotype frequencies for this character also is closely paralleled by a cline in gene frequencies.

5. There is no genetic association between the seedcoat and hairiness characters, and the fact that dense hairiness and non-papillate seedcoats are often associated in nature, as are medium hairiness and papillate seedcoats, cannot be due to linkage or pleiotropic effects.

6. There is some evidence that the rate of germination of hybrid seeds is faster than the rate of germination of selfed seeds.

7. It is shown that hybrid seeds can be recognized as such in the field and an estimate of the proportion of crossing in nature is made, using certain assumptions. This is estimated to vary between 0 and 0·03.

Acknowledgements

I should like to thank Professor W. H. Pearsall for his advice and encouragement during the course of this work. My warm thanks are also due to my husband, fellow research students, and members of the Ministry of Agriculture Advisory Service for help in various ways and to Mr. R. Teasdale for technical assistance. The work described formed part of a Ph.D. thesis presented to the University of London. I am grateful for financial support provided by the Department of Scientific and Industrial Research and by the Central Research Fund of London University.

Literature Cited

Bateman, A. J., 1947: Contamination of Seed Crops I. Insect Pollination. J. Genet., **48**, 257.

Dobzhansky, Th., 1955: A Review of Some Fundamental Concepts and Problems of Population Genetics. Cold Spr. Harb. Symp. quant. Biol., **20**, 1–15.

Hagberg, H., 1953: Further Study on and Discussion of the Heterosis Phenomenon. Hereditas, Lund., **39**, 349–80.

Hayes, H. K., and Garber, R. J., 1927: Breeding Crop Plants. 2nd ed. New York and London, McGraw-Hill, 432 pp.

New, J. K., 1957: A Population Study of *Spergula arvenis*. Ph.D. Thesis, Univ. of London.

—— 1958: A Population Study of *Spergula arvensis*. I. Two Clines and their Significance. Ann. Bot. N.S., **22**, 457.

Stebbins, G. L., Jr., 1950: Variation and Evolution in Plants. New York, Columbia Univ. Press.

Zinger, H. B., 1909: On the Species of Camelina and Spergularia Occurring as Weeds in Sowings of Flax and their Origin. Trudy Bot. Muz. Akad. Nauk. U.S.S.R., **6**, 1–303 (quoted from Stebbins).

Some Aspects of the Biology of *Fusarium oxysporum* Schl. in Soil

BY

DAVID PARK

(*Department of Cryptogamic Botany, University of Manchester*)

With two Figures in the Text

Abstract

From either a mycelial or a conidial inoculum the fungus survived in soil as inactive chlamydospores. The level of its soil population at equilibrium was too low to be studied by dilution plating. Plant materials placed on or beneath the surface of inoculated soil were colonized deeply by the fungus, which produced conidia on them. Dispersal of conidia can occur with water movement in soil, and at right angles to, as well as in the direction of, that movement. No evidence was found of dispersal of the fungus in soil by continuous growth, even over continuous stretches of organic matter. This finding was related to the inability of the fungus to colonize those organic materials that were previously colonized by other organisms from the soil, unless its inoculum potential were greatly augmented. The fungus is thus seen to be a pioneer fungus. The strain used here grew outwards a short distance from colonized organic food bases in the soil, leaving in the soil resting spores which could colonize fresh pieces of organic material subsequently added there. The organism could thus spread by discontinuous growth on successively available, fresh, organic materials.

Introduction

PARK (1958) has shown that a strain of *Fusarium oxysporum* Schl. causing wilt of oil palm can survive on inoculation into alien soils. This fact conflicts with the more commonly found condition whereby the successful introduction of micro-organisms into an alien soil is impossible or difficult and dependent upon a continued modification of the soil conditions (Sanford 1933, Kubiena & Renn 1935, Katznelson 1940). An *a priori* consideration suggests two possibilities for the absence of a given fungus from a particular soil: firstly, the fungus might not have reached that area due to a limited dispersal in space; secondly, assuming that the fungus had reached the area, it may have been unsuccessful there because of unfavourable physico-chemical conditions, or antagonism from more successfully adapted colonizers. The distribution of *F. oxysporum* in areas that have been investigated (Gordon, 1956; Warcup, 1957) indicates that the fungus is widely disseminated, supporting, in the present instance, the likelihood of the second possibility. Arguing against this is the fact that the fungus survives in the alien soil not only passively, but can be active in the competitive colonization of organic substrata. The present paper, extending the study of the activities of *F. oxysporum* in soil, is aimed at resolving this apparently contradictory situation.

[Annals of Botany, N.S. Vol. 23, No. 89, 1959.]

The isolate used throughout is that from the trunk of an oil-palm infected with vascular wilt, and called CW4 in the earlier paper. As in that paper the soil used here is the sandy Styal soil; this had carried *Fagus* and *Betula* scrub, with a ground flora mainly of *Holcus*. No *Fusarium* has been found in this soil by a range of isolation techniques.

Structures Surviving in Soil

The term 'survival' is used in the literature in two senses; it may refer to the retention of viability of cells in the inactive condition, or to a dynamic persistence of a fungus in one place. It was shown (Park, 1958) that *F. oxysporum* survives in inoculated Styal soil under conditions favourable for microbial activity for periods of at least one year, but no observations were made on the morphology of the fungus in soil, or on the means of survival.

Glass threads (Legge, 1952) that had been interpenetrated for 1 week by hyphae of *F. oxysporum* were placed in moist soil in Petri-dishes. At weekly intervals threads were lifted, washed lightly, and stained in hot phenolic rose-bengal for microscopic observation. After 1 week the broad *Fusarium* hyphae were mostly lysed and empty, while numerous mycelial chlamydospores, not present originally, took the stain. Microconidia were fairly frequent. After 2 weeks the broad mycelium was even less common, whereas most of the chlamydospores remained healthy and intact. Microconidia were seen only rarely. After 4 and 6 weeks the only change observed was that the rare microconidia still surviving had become thick-walled (cf. Park, 1957). At observations later than 1 week a type of narrow mycelium was not uncommonly seen: this form also occurred on control threads not bearing *F. oxysporum*. These control threads at no stage showed broad mycelium, or evident chlamydospores or conidia. It is seen, then, that little vegetative mycelium of *F. oxysporum* survived longer than 1 week, the hyphae being soon converted into chlamydospores which appeared to form the main means of survival.

The morphological changes in a conidial inoculum were followed in some detail. Glass threads that had been manipulated in a concentrated aqueous suspension of micro- and macroconidia of the fungus, so that the conidia had become enmeshed in the fibres, were buried in moist Styal soil at 24° C. in Petri-dishes. Periodical observations were made from threads lifted and stained. After 1 week microconidia seemed normal. Complete macroconidia were not seen: all those recognized showed one or two thick-walled cells only. A slender mycelium present was like that in control threads and had no evident connexion with *Fusarium* spores. After 2 weeks many of the microconidia were also thick-walled. After 4 weeks no recognizable conidia were seen, although there was a large number of thick-walled chlamydospore-like bodies of indeterminate origin. Sample threads lifted at 4 weeks, when lightly washed and trailed over the surface of agar plates, deposited bodies of similar form on the surface of the agar. Many of these, on individual transfer to slopes, gave pure fungal cultures, *F. oxysporum* being the commonest isolate. Chlamydospores, then, form the main means of survival of *F. oxysporum*

added to unsterile soil, even when the soil is incubated under conditions favourable for microbial activity, and irrespective of whether the inoculum was largely mycelial or conidial.

Stover (1956*a*) found the level of natural soil populations of *F. oxysporum* too low for it to be accurately studied by a dilution technique. In the present work the population level in artificially inoculated Styal soil was followed by plating. Soil (50 g.) in 100 ml. flasks at 30, 60, and 90 per cent. moisture-holding capacity (m.h.c.) respectively was inoculated uniformly with a standard amount of *F. oxysporum* conidial suspension, and incubated at 23–24° C. Plating by dilution was to 1 in 5,000 in malt agar, the plates being incubated at 25° C. for 7 days. The results (Table 1) show that the decrease in viability of *Fusarium* was least rapid in the dryer soil, but even here the fungus could not, 5 weeks after inoculation, be detected by this method. At each observation soil was used to inoculate droplet plates and *F. oxysporum* continued to be easily found by this method.

TABLE 1

F. oxysporum *Population Level in Inoculated Soil*

As numbers of *F. oxysporum* per g. air dry soil.

	Percentage moisture holding capacity of soil		
	30	60	90
0 days . . .	50,000	47,000	45,000
7 days . . .	5,000	3,750	3,750
11 days . . .	10,000	3,750	1,250
14 days . . .	3,750	8,750	1,250
21 days . . .	4,000	2,000	1,000
28 days . . .	1,250	0	0
35 days . . .	0	0	0

Styal soil that had been inoculated 65 weeks earlier with *F. oxysporum* was tested for the fungus by the two methods. By the dilution method no *Fusarium* could be found, yet droplet platings readily showed that the fungus still survived in the soil. It is thus confirmed that population levels drop below the limit detectable by normal plating by dilution, yet the fungus is still present and easily detectable by the more sensitive and specific droplet-plating method.

Colonization of Plant Materials in Infested Soil

It is known from the author's earlier paper on this subject that the present strain of *F. oxysporum* can competitively colonize sterilized plant materials placed on the surface of inoculated alien soils. Some experiments have been made to determine how far such colonization might be possible under more nearly natural conditions.

Pots of Styal soil inoculated 32 weeks previously with *F. oxysporum*, and incubated at 23–25° C. were used; the population of *F. oxysporum* in this soil was considered to be in equilibrium at a low, quasi-natural level. Pieces of grass leaf and clover stolon, sterilized by propylene oxide, were put in one

series on the surface of the soil, and in the other 5 mm. below the surface. On the third and seventh days observations on colonization were made by surface-sterilizing pieces and plating portions on droplet plates. Observations on conidial production were made by washing pieces in a jet of distilled water and centrifuging the washings. The sediment was examined microscopically for conidia, and also by plating loops on droplet plates. Platings of the pieces of both materials, from both sites, gave abundant *F. oxysporum* colonies. Numerous microconidia and some macroconidia were seen in the sediment from the washings of all pieces, and *F. oxysporum* colonies developed from all the platings of these. It is clear, then, that the fungus colonized sterile pieces of these materials both beneath and upon the surface of soil, and there produced culturally normal spores.

In some repetitions of this experiment the materials were used as collected, i.e. fresh but not sterilized. In each case similar results were obtained, namely colonization by and sporulation of *F. oxysporum* in all pieces both on and under soil after 3 days, indicating that the competitive success of this fungus could extend to non-sterilized, freshly fallen plant materials under natural conditions.

The site of *F. oxysporum* in the colonized clover pieces was determined. Pieces of sterilized clover after 5 days on the surface of *Fusarium*-inoculated Styal soil were surface-sterilized and dissected into (*a*) outer cortex and epidermis, (*b*) inner cortex, and (*c*) vascular tissue and pith, and portions from each of these regions plated separately. All pieces from each region gave *F. oxysporum* showing that the fungus penetrates deeply and is not confined to the surface. A comparison of this finding with the work of Lucas (1955) indicates that *F. oxysporum* exhibits a high degree of competitive saprophytic ability. Waid (1957) makes the point that primary colonizers owe their success to the low amount of competition from other saprophytes, and gives evidence to show that the depth of colonization of such organisms is low. *F. oxysporum* appears to be unusual in being an early colonizer that penetrates deeply.

Dispersal by Continuous Growth

Glass tapes (Legge, 1952), 5·5 cm. × 5 mm., colonized for about 1 cm. at one end by *F. oxysporum*, were partially cut to show the limit of *Fusarium* growth, and were then buried in moist Styal soil in Petri-dishes incubated at 23–25° C. Each week six tapes were lifted, cut into 5-mm. zones, and portions plated, the originally colonized zone being classed as zone 0, and subsequent 5 mm. zones as 1, 2, and so on, in order. The results (Table 2) might be interpreted as showing some initial growth, less than 5 mm., followed by a cessation. It is also possible that the *Fusarium* in zone 1 originated from conidia that had migrated laterally from zone 0.

A similar uncertainty applies to another experiment in which 0·2-ml. lots of a spore suspension were put down in localized, marked areas on the surface of dishes of Styal soil. Here, observations by weekly platings of portions of soil taken at 0·5 cm. intervals from the edge of the inoculated region showed

that *F. oxysporum* survived in that region, and had spread after 1 week to the 0–5 mm. region, but no further in 8 weeks. Despite the uncertainty in these two experiments as to whether growth or spore migration had occurred, both observations rule out the occurrence of any extensive growth through the non-sterile soil matrix.

TABLE 2

Growth of F. oxysporum *along Glass Tapes in Soil*

Replicates showing *F. oxysporum* out of the total of six.

	5 mm. zones			
	0	1	2	3
1 week	6	0	0	0
2 weeks	6	4	0	0
3 weeks	6	3	0	0
8 weeks	6	4	0	0

There is the possibility that decomposable material in soil might act as a substratum along which continuous growth could occur. Lengths of dead root material less than 1 mm. in diameter were taken out of Styal soil that had been collected for longer than 3 weeks. Pieces of these trimmed to about 1 cm. long were laid end to end, in contact, in chains of five on the surface of Styal soil incubated at 23–24° C. One end of each chain was inoculated by putting an agar disc of *F. oxysporum* on the end member. Observations at intervals by surface-sterilizing and plating showed that *Fusarium* did not enter any of the root pieces, not even the one touching the agar inoculum. It is thus seen that such old organic materials are not, in the form in which they occur in soil, able to serve naturally to disperse *F. oxysporum* through soil by allowing its growth.

Dispersal by Growth Outward from a Food Base

Even in the absence of continuous growth in soil, another type of dispersal by growth must be considered, namely one effected by a series of short bursts of growth, each ephemeral and originating from a colonized food base. The food base used here was potato discs, 3·5 mm. diam. × 2 mm., colonized by *F. oxysporum* in pure culture for 5 days. Sterile potato discs placed at successive 5 mm. intervals from the food base-inocula served to determine effective colonization after growth outwards from the inoculum. One series of such sets of discs was put on the surface of soil. In another series the pieces were 4 to 5 mm. below the surface of the soil. Dishes were incubated at 23–24° C. Weekly observations by droplet plating portions of discs showed that *F. oxysporum* survived in the inoculated disc but did not colonize any of the other discs in 4 weeks. It should be noted that for the fungus to have given a positive result two properties would have been necessary: firstly, the ability to grow through the soil, and, secondly, the ability to colonize the new discs. The negative result could have been due to failure in either of these phases.

A test for outward growth from the same sort of food base on the surface of Styal soil was made by putting colonized discs on the 'slide area' of a soil dish and observing at weekly intervals by contact slides (Park, 1955). By this direct method it was found that *F. oxysporum* did grow outward over the soil. At 1 week hyphae, bearing chlamydospores and associated with numerous *Fusarium* microconidia and some macroconidia, were identified as far as 3 mm. distant from the inoculum. After 2 weeks a distance of 4 mm. was the greatest on which reliance was placed: at this time stained hyphae were less common, and macroconidia were mostly present as 1-celled pieces. At the final observation (4 weeks) very little mycelium remained away from the discs, although chlamydospores and microconidia-like structures were seen up to 3 mm. from the edge of the inocula. Droplet platings of crumbs of soil at millimetre intervals from the edges of discs at the 4-week stage gave *F. oxysporum* as the dominant fungus in the 1 to 4 mm. region. The fungus was also obtained readily from points 5 and 6 mm. distant. Thus *F. oxysporum* does grow outward at least 6 mm. from such a food base, and leaves viable propagules after the vegetative mycelium has decomposed. It can, therefore, be assumed that the fungus grew to the uncolonized potato discs in the previous experiment, but that it was unable to colonize them under the conditions there prevailing. This deduction appears to conflict with earlier statements as to the high competitive saprophytic ability on the part of the fungus. The situation will be made clear in some of the following pages.

The foregoing demonstration that *F. oxysporum* can spread outward from a colonized food base is not directly related to natural conditions, since the food base used was colonized in pure culture. Repetitions were made, using for the food base pieces of propylene oxide-sterilized clover stolon colonized for 4 days on Styal soil inoculated 36 weeks previously with *F. oxysporum*. Sample pieces showed that all were colonized by *F. oxysporum* although indigenous soil saprophytes were also present. Observations from the contact slides prepared showed that *Fusarium* microconidia had been produced up to 3·5 mm. away from the edges of the pieces during the 4 weeks. At 4 weeks *F. oxysporum* was common in platings of soil from distances up to 4 mm. away from the inoculum. The fungus, then, can spread outward for small distances from a food base colonized under near-natural conditions.

Spread of the fungus from a food base beneath the surface of soil was examined by inserting pieces of clover, colonized as above, each into the fibres of a length of sterile glass tape, and burying the tapes at a depth of 5 mm. in soil. At weekly intervals tapes were lifted and cut into millimetre bands starting from the edge of the food base. Portions of the strips were plated on droplets. After 1 week the fungus had spread to strip number three (2–3 mm. from the food base) in all tapes. Subsequent observations to $4\frac{1}{2}$ weeks showed similar results, with most of the tapes indicating spread to 2–3 mm., and not more than 10 per cent. indicating spread to the 3–4 mm. band.

It is seen from the experiments in this section that *F. oxysporum* can grow outward for limited and small distances from a food base. Natural plant

material colonized under near-natural conditions is effective as a food base in such growth, and the mechanism, although not so spectacular as a continuous spread, could be ecologically important in increasing the range of the fungus.

Spore Dispersal in Soil

Experiments described earlier in this paper indicated that some dispersal of *F. oxysporum* might have been due to migration of spores in moist soil. This series of experiments was designed to investigate this possibility. Since

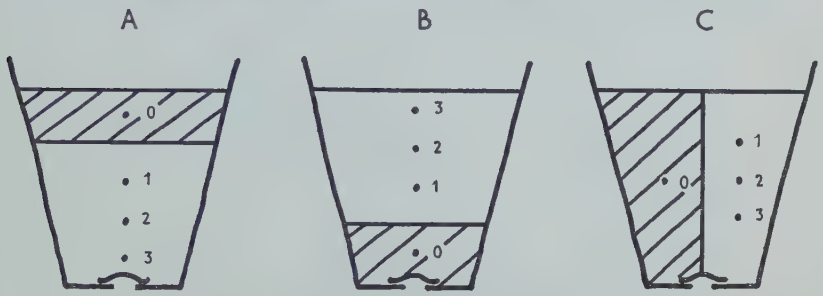


Fig. 1. Arrangement of soils and sampling sites in spore-migration experiments:
infested soil—shaded,
uninfested soil—unshaded.

the work was done Rishbeth (1957) has reported some experiments that show that a conidial suspension of *F. oxysporum* added to soil can be moved downwards by a water current.

Here, the movement of the fungus from soil infested with *F. oxysporum* into uninfested soil was followed. The infested soil was prepared by mixing into air-dry Styal soil 2 per cent. of sterilized chopped grass leaf on which the fungus had grown for 4 weeks. Three series of pots were set up containing infested and uninfested soil in separate parts, as shown in Fig. 1. Two types of irrigation were followed in each series, (i) pots watered daily by sprinkling from above, (ii) pots watered from below by periodically standing in 2 cm. of water until moisture appeared at the surface. The subsequent distribution of *F. oxysporum* was examined by splitting the soil block from each pot vertically, and transversely to the junction of the two soil types. Samples from the originally infested soil and from three points in the originally uninfested soil were plated on droplets. The samples in series *A* and *B* were taken at 1-cm. intervals from the junction of the soils, while in series *C* the three sampling sites were all 1 cm. from the junction and 1 cm. from each other. Relative positions of these sampling sites are illustrated in Fig. 1. Results of the platings made after 9 days are given in Table 3. After 3 and 4 weeks further observations were made on *A* (ii), *B* (i), and *C* (ii); no change had occurred in *A* (ii); *B* (i) showed *F. oxysporum* in two out of three pots at site 1, but still none at sites 2 and 3; *C* (ii) gave *F. oxysporum* at all sites in the 3 and 4 weeks' observations. It is clear from this that infestation of uninoculated soil from

inoculated soil under the influence of a vertical slow water current occurs extensively only in the direction of the current, whether this is with or against gravity. Lateral infestation, i.e. dispersal at right angles to the current, is possible; the fact that this was slower here under the influence of an upward flow than a downward one is probably not significant since rate and amount of flow were not necessarily comparable between the two methods of irrigation.

TABLE 3

Movement of F. oxysporum *after Nine Days in Irrigated Soil (See Fig. 1)*

Figures are the numbers of triplicate pots showing *F. oxysporum* at the site.

Sample site	Series and watering treatment					
	A(i)	A(ii)	B(i)	B(ii)	C(i)	C(ii)
0	3	3	3	3	3	3
1	3	0	1	3	3	0
2	3	0	0	3	3	0
3	3	0	0	3	3	0

Pots set up in the same way as the above were used to examine the movement of propagules from infested to uninfested soil in moist soil but without any water flow. Saturated soil was used, and the filled pots were kept in Meilleur jars in an atmosphere of high humidity, so that the soil remained near saturation point throughout. Platings from the originally uninfested soil of all pots gave no *F. oxysporum* even after 6 weeks. This is of interest since it enables one, in the previous experiment, to eliminate the possibility of mycelial growth rather than propagular migration. Furthermore, it shows that the transport of the propagules is dependent upon active water flow, even where transport is in a direction at right angles to that flow.

A more detailed observation on the direction of movement in relation to that of the water flow was made by putting inoculated soil in a restricted position in relation to the uninfested soil. The infested soil was retained in a cylindrical shape (1 cm. diam. × 1 cm. high) with the aid of strips of glass, and was packed tightly on all sides with uninfested soil. Different series of pots were watered from above and from below as in the first experiment in this section. After 3 weeks the soil blocks were split vertically, the cut surfaces marked into grids of 1 cm. squares, and samples from the centre of each square droplet-plated. Figs. 2*a* and 2*b* show representative results of the distribution of *F. oxysporum* within the soils. It is clear that the distribution follows a cone shape with the greatest lateral spread near the source of the propagules. This type of distribution is that given by particulate substances when run through a porous substratum in a current of non-solvent liquid. Fig. 2*c* shows a pattern obtained by running in water fine carbon particles in filter paper. It is probable then that in a current of water lateral flow of *F. oxysporum* propagules in soil may be considerable, especially near to the inoculum.

An important conclusion to be drawn from this section is that dispersal of the fungus in any soil subject to moisture fluctuations may be more effectively brought about by passive transport of propagules than by active mycelial growth, even where a high level of food bases may be present.

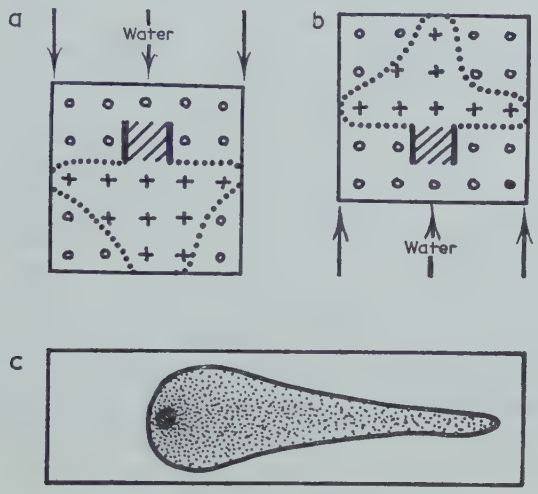


Fig. 2. *a* and *b*. Distribution of spores of *F. oxysporum* in soil under the influence of water movement.

Cross-shading = originally infested soil,
o = no *Fusarium* at end of test,
+ = *Fusarium* present at end of test.

c. Distribution of carbon particles from the spot through filter paper by water flow.

Dispersal by Growth from Food Base to Food Base, and Effect of Pre-colonization of Organic Material

It was earlier shown that *F. oxysporum* is a highly successful competitive colonizer of fresh organic material. Yet apparent anomalies are outstanding, namely the lack of continuous growth along root pieces in soil, and the inability to colonize the potato discs in soil in an earlier experiment. It was decided therefore to investigate more closely some relationships in the colonization by *F. oxysporum* of one piece of organic material from another.

Pieces of propylene oxide-sterilized plant material, each 5 mm. long, were laid on soil in chains of four, touching end-to-end. A fifth piece, colonized for 5 days in *F. oxysporum*-inoculated Styal soil, was laid touching one end member of each chain. Duplicate series were run for pieces on the surface and for those 5 mm. below the surface. In both, grass and clover were used. Dishes were incubated at 23–24° C. Periodical observations were made by lifting each chain, designating the inoculum piece as 0, and the remainder as 1, 2, 3, and 4, successively, and surface-sterilizing and plating the pieces. The results after 3 days were the same for both types of plant material, and

both on and below the soil surface, namely, only the piece actually in contact with the inoculum became colonized by *F. oxysporum*, the remainder having only fungi indigenous to Styal soil. Observations were continued to 4 weeks without showing any further spread of *Fusarium* along the chain.

In some repetitions of this experiment the inoculum (segment 0) was a piece of plant material colonized by *F. oxysporum* in pure culture. The results were again the same, the first segment only becoming colonized during the whole 4-week period. Thus the fungus spreads to the first segment in less than 3 days, but in ten times as long still gets no further than this one 5-mm. segment.

A check was made that *F. oxysporum* is able to spread continuously along plant pieces when colonizing from the mixed inoculum of *Fusarium*-infested Styal soil. Propylene oxide-sterilized pieces of clover stolon about 4·5 cm. long were stood vertically with one end in such soil at 24–26° C. At intervals pieces were lifted, surface-sterilized, and cut into 5 mm. segments, the buried part being segment 0, and the remainder segments 1, 2, 3, &c. Samples were droplet-plated to determine *F. oxysporum* colonization. The results (Table 4) show that *F. oxysporum* can spread along clover from the inoculated soil, and eliminate the possibility that the *Fusarium* is overtaken and inhibited by some other colonizer from the soil.

TABLE 4

Spread of F. oxysporum *along Clover in Air, Colonized from* Fusarium-*inoculated Soil*

	Numbers of segments, out of ten replicates, showing *F. oxysporum*. 5 mm. segments								
	0	1	2	3	4	5	6	7	8
2 days . . .	10	10	7	0	0	0	0	0	0
5 days . . .	10	10	10	10	9	1	0	0	0
7 days . . .	10	10	10	10	10	10	10	7	5

There remains the possibility that in the chains of segments laid on the surface of the soil the later segments in the chains become colonized directly from the soil by other organisms, one or more of which prevents subsequent colonization by *F. oxysporum*. Propylene oxide-sterilized pieces of clover and grass were added to uninoculated Styal soil held at 23–24° C., and, each day, batches of twenty each were transferred to *F. oxysporum*-inoculated Styal soil, where they were left for 4 days before being surface-sterilized and samples droplet-plated. The two results for each material (Table 5) are the extremes of the range obtained in several repetitions. Three days' contact between a sterile piece of plant material and fresh Styal soil, then, allowed in all cases sufficient pre-colonization to prevent entry of *F. oxysporum*. This fungus thus appears in the role of a primary colonizer, a pioneer fungus whose success is confined to early stages in the ecological succession on decomposing

materials, in the later stages of which it is prevented from entering by antagonisms from other organisms.

TABLE 5

Effect of Pre-colonization in Uninoculated Soil on Colonization of Plant Material by Fusarium

Numbers of pieces, out of ten, showing *F. oxysporum*. The two sets of figures for each material show the extremes of the results obtained in different trials.

Number of days' pre-colonization	Material: clover		Material: grass	
0	10	10	10	10
1	3	8	4	6
2	0	3	0	2
3	0	0	0	0

The foregoing result explains the lack of continuous growth of *F. oxysporum* along a chain of organic pieces that is added to soil at one time. If such pre-colonization, allowing antagonism, were the sole reason for the lack of spread of *F. oxysporum* through a chain of organic pieces on soil it would be expected that continuous spread should occur along *successively* added pieces of material, each being sterile when put into contact with the segment previously colonized by the fungus. A single piece (segment 0) of clover stolon 5 days colonized in *F. oxysporum*-inoculated Styal soil was put on the surface of fresh Styal soil and left for 7 days. Then, segment 1, a 1-cm. piece of propylene oxide-sterilized stolon, was added to the soil just touching segment 0: this was left for a further 7 days, after which segments 2 and 3, again each with a 7-day interval between, were added. After a final 7 days incubation, the segments were surface-sterilized and portions plated on droplets. In a second series the same procedure was followed but a 2 mm. gap was left between the adjacent segments in each chain. Results of the plating (Table 6) make it clear that

TABLE 6

Growth of F. oxysporum *along Successively Added Segments of Clover Stolon*

(Number of test pieces with *Fusarium* out of six.)

	Segments touching				Segments with 2 mm. gap			
Segment no.	0	1	2	3	0	1	2	3
No. of pieces with *Fusarium*	5	5	5	4	6	3	1	0

F. oxysporum was able to grow successively along material in soil under these conditions when the pieces were touching. The results from the segments separated by the 2 mm. gap show that the chances of colonization of the adjacent pieces are reduced, although some success was shown. A repetition of the experiment with freshly collected clover stolon, not sterilized, gave similar results, indicating that this type of dispersal could occur under conditions approaching closely to natural.

The data obtained so far show that *F. oxysporum* can competitively colonize fresh organic materials, but that if these are already colonized for at least three days by indigenous organisms from Styal soil then *F. oxysporum* is unable to enter. These conclusions were tested further in the following way. Samples of soil were collected and stored for 2 weeks. This allowed any organic material that had been killed in collection to become colonized from the soil. The soil was then inoculated with conidia of *F. oxysporum*, and incubated in an unheated greenhouse under conditions of fluctuating moisture for 9 months. At the end of this time pieces of the organic materials from the soil were lifted, surface-sterilized, and portions plated on droplets. *F. oxysporum* was found to have colonized 1–2 per cent. of such pieces. Thus, contrary to earlier findings, it appears that some organic materials assumed to be already colonized by soil saprophytes had subsequently become colonized by *F. oxysporum*. It is suggested that this may have occurred under some specially favourable conditions, since the conditions of incubation included a wide range of temperatures and moisture levels.

In a further test, 3-day pre-colonized pieces of clover and grass were put on the surface of moist, sterile sand cultures, and a *F. oxysporum*-colonized piece of the same type of organic material brought into contact with each piece. The inoculum pieces were colonized in one of two ways: (*a*) colonized for 3 days in *F. oxysporum*-inoculated Styal soil, (*b*) colonized for 3 days in a pure culture of *F. oxysporum* in sand and water. A typical result of colonization of the test pieces after 4 days is given (Table 7) and shows that *Fusarium*

TABLE 7

Colonization of Pre-colonized Materials from Organic Pieces Colonized by F. oxysporum *in Different Ways*

Figures show number of test-pieces, out of ten, colonized.

	Material	
	clover	grass
From *Fusarium*-inoculated soil . . .	0	0
From *Fusarium* sand culture . . .	8	10

can enter a large proportion of the pre-colonized pieces when it grows from the food base occupied solely by *F. oxysporum*, in other words, under conditions of high inoculum potential. The presence of the fungus then in a minority of pieces in the previous test may be explained as due to the existence, at some time during the incubation of the soils, of an unusually high inoculum potential of *F. oxysporum*. Under most normal conditions the presence of the Styal microflora established in organic material is sufficient to keep out *F. oxysporum*.

DISCUSSION

In normal soil without fresh decomposable material the fungus survived in an inactive condition in the form of chlamydospores, irrespective of whether the inoculum was mycelial or conidial. It is noted that Warcup (1957) was

able to isolate *F. oxysporum* from a soil by the method of hyphal isolation, yet the same author makes the point that isolation in this way does not in itself necessarily indicate current activity of the fungus. Moreover, in the same paper he states that the origin of most *Fusarium* colonies on dilution plates is either hidden by humus particles or is from chlamydospores. Population levels of the fungus at equilibrium with the soil under more or less natural conditions were, in agreement with the findings of Stover (1956*a* and *b*), too low to be examined in any detail by plating by dilution, yet were readily isolated by droplet-plating. Rishbeth (1955) had earlier found that the population of *F. oxysporum* f. *cubense* added to soil in large quantities declined to a level not detectable by standard plating methods, but which could continue to cause disease.

Passive dispersal of the fungus in soil by migration of spores under the influence of water flow could be important in natural conditions of fluctuating soil moisture. It is known that flood and irrigation water can serve as most effective agents of other soil-borne pathogens, including some wilt fusaria (Bewley and Buddin, 1921; Curzi, 1927; Smith, 1932; Crawford, 1934; Brown and Butler, 1936; Cooke and Kabler, 1957). Also percolating water may carry fungal spores in a downward direction through soil (Burges, 1950). Rishbeth (1957), extending this mechanism to *F. oxysporum* f. *cubense*, found that the amounts of water produced by rain would be sufficient to carry spores from a suspension on the surface of the soil to the root system of the suscept. The present paper shows that under the sort of fluctuating moisture levels found in natural soils spores can also be carried in a horizontal as well as in a vertical direction. Such migration might easily have a significance for dispersal of the fungus greater than that of mycelial growth, even in a soil containing much organic material. Prendergast (1957) has drawn attention to the fact that the Nigerian region of most serious oil-palm wilt is one which has great fluctuations of the water table.

It was stated in an earlier paper (Park, 1958) that *F. oxysporum* has a high degree of competitive saprophytic ability and is considered to be a soil inhabitant. Stover and Waite (1954), however, claim that the strain of *F. oxysporum* studied by them is a weak colonizer of organic materials in soil. The present work shows that the high degree of competitive saprophytic ability of *F. oxysporum* is expressed under certain limited conditions, and that the fungus is successful as a colonizer in soil of dead organic materials only in the very early stages of their decomposition. The fungus may therefore be classified as a 'sugar fungus' (Burges, 1939), although the term *pioneer* fungus has been preferred here. The fungus shows some success in competition with saprophytes indigenous to the soil, and also exhibits some parasitic properties; its status, therefore, lies somewhere between that of typically saprophytic 'sugar fungi' and that of ecologically obligate parasites of the *Ophiobolus graminis* type, which was described as a pioneer fungus by Garrett (1952).

The disagreement between the results reported here and those of Stover and Waite (1954) with regard to the competitive saprophytic ability of *F.*

oxysporum may be explained as easily on the grounds of the different conditions of presentation of the substratum as on the basis of strain differences. It is clear that the designation of a fungus as a soil inhabitant or a soil invader is only a first step in an ecological classification of the fungus, and it is insufficient merely to say that a fungus is a strong or a weak competitive saprophyte. It is necessary to know under what conditions any competitive saprophytic ability is expressed. The purpose of an autecological study is to determine the status held by the organism in soil, and the conditions that affect its success in its ecological niche.

The strain of *F. oxysporum* used here, then, shows a high degree of competitive saprophytic ability when colonizing sterilized or freshly dead organic materials. However, previous colonization of the materials for 2 or 3 days is normally sufficient to prevent entry of the fungus. The work of Bega (1954) contains a parallel situation in which colonization of wounded plant parts by *F. solani* prevented subsequent infection by *F. oxysporum*. Conversely, Subramanian (1950) found that the wilt *Fusarium* that he studied was able to colonize plant materials that were already colonized by other micro-organisms. These conflicting results can be reconciled in terms of the inoculum potential of the colonizing fungus. It was found in the present study that by greatly increasing the inoculum potential of *F. oxysporum* this fungus was enabled to overcome the antagonism of organisms resident in the organic material; Stover and Waite (1954) similarly found increased colonization under such conditions. Moreover, the foregoing observations of Subramanian were, in fact, made in wilt-sick soil in which the fungus is understood to have a high inoculum potential.

Literature Cited

Bega, R., 1954: Biological Control of *Fusarium* Wilt of Sweet Potato. Phytopathology, **44**, 482.

Bewley, W. F., and Buddin, W., 1921: On the Fungus Flora of Glasshouse Water-supplies in Relation to Plant Diseases. Ann. appl. Biol., **8**, 10–19.

Brown, J. G., and Butler, K. D., 1936: Sclerotiniose of Lettuce in Arizona. Tech. Bull. Ariz. agric. Exp. Sta., **63**, 475–506.

Burges, A., 1939: Soil Fungi and Root Infection. Broteria, **8**, 64–81.

—— 1950: The Downward Movement of Fungal Spores in Sandy Soil. Trans. Brit. mycol. Soc., **33**, 142–7.

Cooke, W. B., and Kabler, P. W., 1957: Plant Disease Fungi in Sewage Polluted Water. Publ. Hlth. Rep., Wash., **72**, 651–4.

Crawford, R. F., 1934: The Etiology and Control of Chile Wilt, Produced by *Fusarium annuum*. Tech. Bull. New Mexico agric. Exp. Sta. **223.**

Curzi, M., 1927: L'eziologia della 'cancrena pedale' del *Capsicum annuum*. Riv. Pat. Veg., **17**, 1–19. Abs. in Rev. appl. Mycol., **6**, 333.

Garrett, S. D., 1952: The Soil Fungi as a Microcosm for Ecologists. Sci. Progr., Twent. Cent., **40**, 436–50.

Gordon, W. L., 1956: The Occurrence of *Fusarium* Species in Canada. V. Taxonomy and Geographic Distribution of *Fusarium* Species in Soil. Canad. J. Bot., **34**, 833–64.

Katznelson, H., 1940: Survival of Microorganisms Inoculated into Soil. Soil Sci., **49**, 283–93.

Kubiena, W., and Renn, C. E., 1935: Micropedological Studies of the Influence of Different Organic Compounds upon the Microflora of the Soil. Zbl. Bakt., 2, **91**, 267–91.

Legge, B. J., 1952: Use of Glass Fibre Material in Soil Mycology. Nature, Lond., **169**, 759.

Lucas, R. L., 1955: A Comparative Study of *Ophiobolus graminis* and *Fusarium culmorum* in Saprophytic Colonization of Wheat Straw. Ann. appl. Biol., **43,** 134–43.

Park, D., 1955: Experimental Studies on the Ecology of Fungi in Soil. Trans. Brit. mycol. Soc., **38,** 130–42.

—— 1957: Behaviour of Soil Fungi in the Presence of Bacterial Antagonists. Ibid., **40,** 283–91.

—— 1958: The Saprophytic Status of *Fusarium oxysporum* Schl. Causing Vascular Wilt of Oil Palm. Ann. Bot., n.s., **22,** 19–35.

Prendergast, A. G., 1957: Observations on the Epidemiology of Vascular Wilt Disease of the Oil Palm (*Elaesis guineensis*, Jacq.). J. W. Afr. Inst. Oil Palm Res., **2,** 148–75.

Rishbeth, J., 1955: *Fusarium* Wilt of Bananas in Jamaica. I. Some Observations on the Epidemiology of the Disease. Ann. Bot., n.s., **19,** 293–328.

—— 1957: *Fusarium* Wilt of Bananas in Jamaica. II. Some Aspects of Host-parasite Relationships. Ibid., **21,** 215–45.

Sanford, G. B., 1933: Some Soil Microbiological Aspects of Plant Pathology. Sci. Agric., **13,** 638–41.

Smith, F. E. V., 1932: Panama Disease of Bananas in Jamaica. Microbiol. Bull. Dep. Agric. Jamaica, **1.**

Stover, R. H., 1956*a*: Behaviour of *Fusarium oxysporum* f. *cubense* in Different Soils and Soil Extracts. Phytopathology, **46,** 27–28.

—— 1956*b*: Studies on *Fusarium* Wilt of Bananas. I. The Behaviour of *F. oxysporum* f. *cubense* in Different Soils. Canad. J. Bot., **34,** 927–42.

—— and Waite, B. H., 1954: Colonization of Banana Roots by *Fusarium oxysporum* f. *cubense* and other Soil Fungi. Phytopathology, **44,** 689–93.

Subramanian, C. V., 1950: Soil Conditions and Wilt Disease in Plants with Special Reference to *Fusarium vasinfectum* on Cotton. Proc. Ind. Acad. Sci., b, **31,** 67–102.

Waid, J. S., 1957: Distribution of Fungi within the Decomposing Tissues of Ryegrass Roots. Trans. Brit. mycol. Soc., **40,** 391–406.

Warcup, J. H., 1957: Studies on the Occurrence and Activity of Fungi in a Wheat Field Soil. Ibid., **40,** 237–59.

Physiological and Ecological Studies in the Analysis of Plant Environment

XI. A Further Assessment of the Influence of Shading on the Growth of Different Species in the Vegetative Phase

BY

G. E. BLACKMAN AND J. N. BLACK[1]

(*Department of Agriculture, Oxford*)

With nine Figures in the Text

ABSTRACT

In a further analysis of the effects of varying light intensity on growth and development in the vegetative phase the reactions of thirteen herbaceous species have been recorded. In some experiments the degree of shading has been extended to 0·055 daylight, a level near or below the compensation point.

For *Lathyrus maritimus*, *Trifolium pratense*, and *Vicia faba*, the net assimilation rate is directly related to the logarithm of the light intensity, but for *Helianthus annuus*, *T. repens*, *T. hybridum*, *Medicago sativa*, *Phaseolus multiflorus*, and *Lolium multiflorum* the relationship, though curvilinear, is not logarithmic. It is concluded that for all species the assimilation rate of unshaded plants was limited by light even though in high summer the recorded light energy between 4,000–7,000 Å averaged 1,900–2,200 foot-candles.

For all these species between daylight and 0·12 daylight the leaf-area ratio rises as the intensity decreases and in general the trend is logarithmic. When the degree of shading is increased to 0·055 daylight the logarithmic relationship still holds for *L. maritimus* and *V. faba* though this level is below the compensation point. For other species, such as *P. multiflorus* and *H. annuus* the trend may be reversed below 0·12 daylight and the ratio then falls.

When the light intensity is reduced from daylight to 0·5 daylight, then for the species already cited and for *Lolium perenne*, *Phleum pratense*, and *Festuca pratensis*, the relative growth-rate is invariably depressed. At 0·055 daylight the relative growth-rate never exceeded 1 per cent. per day. For *L. perenne*, *P. pratense*, and *Dactylis glomerata* the reactions to shading of 'hay' and 'grazing' strains were different.

The ecological and physiological implications of these findings are discussed.

INTRODUCTION

IN two previous papers of this series (Blackman and Wilson, 1951, *a* and *b*) an analysis was made of the effects of shading on the growth in the early vegetative phase of *Helianthus annuus* and nine other species. Employing a range of screens which reduced in steps the light level down to either 0·24 or 0·12 daylight it was established that the net assimilation rate (rate of gain in dry weight of whole plant per unit area of leaf) was dependent on the amount of light received, while in contrast the leaf-area ratio (total leaf area/plant

[1] Present address: Waite Agricultural Research Institute, University of Adelaide.

[Annals of Botany, N.S. Vol. 23, No. 89, 1959.]

weight) rose as the level of shading was increased. When the seasonal conditions were favourable for growth it was found that these positive and negative relationships were both approximately logarithmic. Since the relative growth-rate (rate of gain in dry weight of whole plant) is the product of the leaf-area ratio and net assimilation rate it follows that the influence of shading on the rate of growth is the resultant of the opposing effects of these two components. For some of the species examined, notably *Geum urbanum*, the decrease in the net assimilation rate was more than counterbalanced by the increase in the leaf-area ratio and the relative growth-rate was maximal at half daylight. In contrast for some other species, such as *Tropaeolum majus*, *Trifolium subterraneum*, and *Pisum sativum*, the fall in assimilation was greater than the corresponding rise in the leaf-area ratio and it was deduced that the optimal level for growth was above that of normal daylight. There was also a third group of plants for which the optimal light intensity lay between three-quarters and full daylight.

For only one of the species (*Vicia faba*) was the net assimilation rate reduced to a very low value by the deepest level of shade investigated and it was not therefore possible to establish whether the observed reactions to light would be modified at levels in the neighbourhood of the compensation point. This aspect has now been further studied. There was also some indication from the previous experiments that herbaceous legumes might be particularly light-demanding and so additional species have now been examined. Lastly, most of the previous experiments had been carried out on dicotyledenous plants and it seemed desirable to determine the effects of shading on the growth of a number of graminaceous species.

Experimental Methods

The general experimental procedure has been described in detail in previous papers, e.g. Blackman and Wilson (1951*b*) and Blackman, Black, and Kemp (1955), and so will only be summarized here. Seed of the various species was sown in pots (25 cm. diam.) in a mixture of sand and soil to which a fertilizer containing nitrogen, phosphorus, and potassium, was added. After emergence the seedlings were thinned to a small number per pot so that they should not shade each other to any appreciable extent. At the beginning and end of each experiment the shoots were cut off at ground level, the leaves separated from the stems, and the roots recovered by washing the contents of the pot through wire sieves. The stems, leaves, and roots were dried separately (100° C. for 24 hours) and then weighed. Immediately after the leaf blades were removed, a sub-sample from each pot was 'blue printed' before being dried and weighed, and the areas subsequently determined with a planimeter.

The method of shading was to suspend over groups of pots light wooden frames (1·5 × 1·2 m.) covered with sheets of perforated zinc with additional vertical side pieces to prevent undue lateral illumination. By varying the size and number of holes in the sheet the proportion of daylight reaching the plants was so reduced that they received 0·5, 0·24, 0·12, and 0·055 daylight.

In some experiments there was an additional screen of woven wire with a transmission factor of 0·76.

During the two years in which the experiments were undertaken the total light energy between 4,000 and 7,000 Å received per day was measured by means of a specially constructed integrating light recorder, of which details are to be found in Blackman, Black, and Martin (1953). At that time there were no facilities available for the accurate calibration of the instrument and it had perforce to be calibrated against a tungsten filament lamp conforming to the standard of the National Physical Laboratory. Subsequent comparisons against a Kipp solarimeter have demonstrated its reliability and freedom from temperature drifts. In this investigation the daily light energy received has been expressed as foot-candles × hour.

Experimental Results

1. *The influence of shading on the growth of Helianthus annuus*

Four experiments on *H. annus* were undertaken at different periods of the growing season, in September 1950 and in May, June, and June–July 1951. As far as possible each experiment was started as soon as the plants of the semi-dwarf variety (Pole Star) had reached the stage when the third pair of true leaves were visible. The number of plants per pot was always reduced to six while, to minimize errors, the replication was high—eight- to tenfold according to the experiment. In three of the experiments the degree of shading extended to 0·055 daylight, at which level little or no growth was expected.

The results of the four experiments have been grouped together in Figs. 1 and 2. Considering first the net assimilation rate, inspection shows that over this wide range of shading the relationship with the logarithm of light level is not linear but curvilinear. This was confirmed in each experiment by statistical analysis and the appropriate equation of closest fit has therefore been fitted. Turning to the changes in the leaf-area ratio, in experiments 3 and 4 (Fig. 2) there is an inverse linear relationship with the logarithm of the light level, but in experiment 1 (Fig. 1*a*) the relationship is curvilinear. This curvilinear trend is also suggested by the data of experiment 2 (Fig. 1*b*) but the deviation from linearity is not statistically significant.

Since the relative growth-rate is the product of the net assimilation rate and the leaf-area ratio the equation linking the relative growth-rate with the logarithm of light intensity can be derived by multiplying the corresponding equations for the two components. Unless these pairs of equations fit the data closely the errors in the derived equation (dotted line), which may involve terms up to the fourth power (as in experiment 1), will be magnified. It was not therefore unexpected to find that the fits were only approximate and that more satisfactory results could be obtained by calculating the regressions of closest fit. In fact statistical analysis revealed that for each experiment the departure from a logarithmic relationship was not significant.

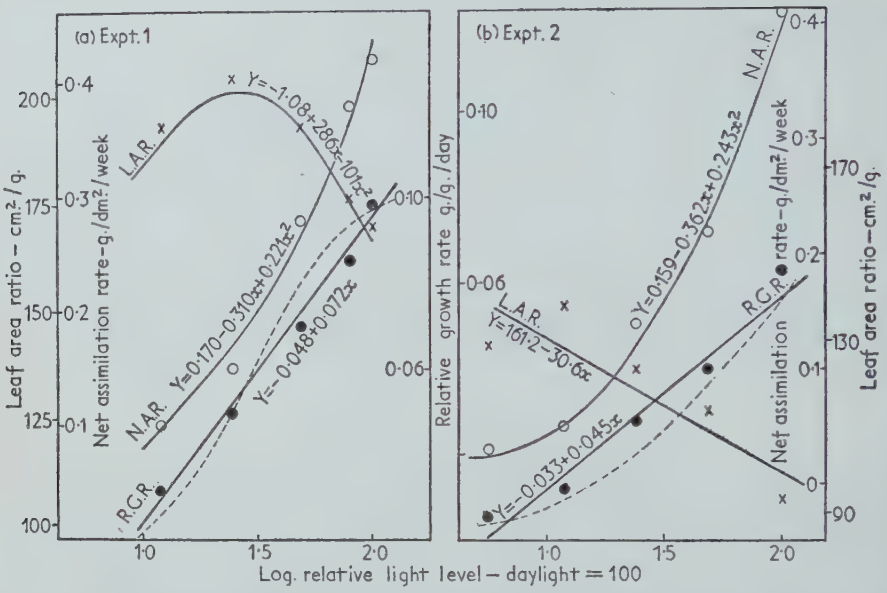


FIG. 1. *Helianthus annuus*. The effects of varying light level on the net assimilation rate (NAR), leaf-area ratio (LAR) and relative growth-rate (RGR). The mean diurnal light received by the unshaded plants in experiments 1 and 2 was 18,240 and 26,820 foot-candle hours respectively.

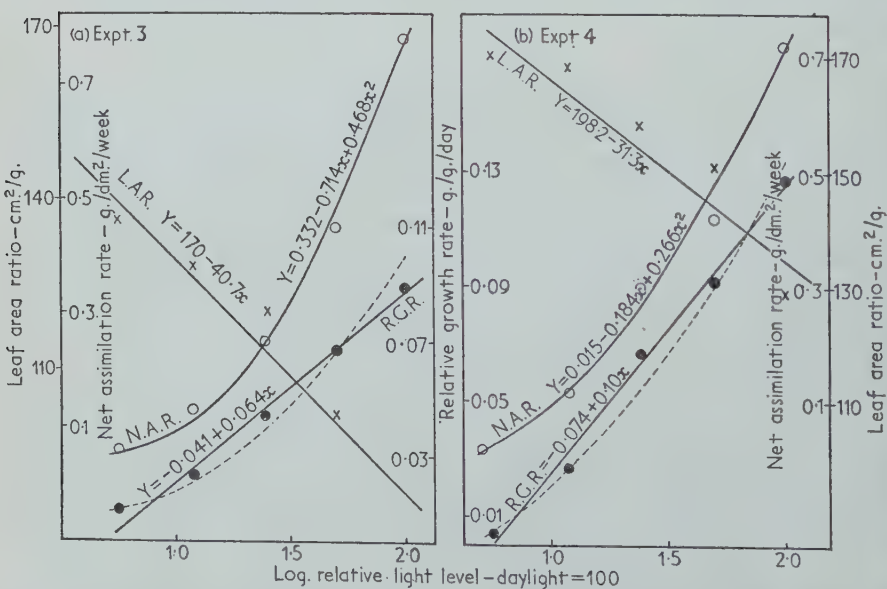


FIG. 2. *Helianthus annuus*. The effects of varying light level on the net assimilation rate (NAR), leaf-area ratio (LAR) and relative growth-rate (RGR). The mean diurnal light received by the unshaded plants in experiments 3 and 4 was 32,070 and 36,960 foot-candle hours respectively.

2. *The influence of shading on the growth of some leguminous species*

Amongst the species selected for investigation were *Lathyrus maritimus*, found on littoral shingle banks and *Medicago sativa* originating from semi-arid areas of the warm temperate zone. Seed of *L. maritimus* was sown in pots on July 7, 1951, and the experiment started 33 days later, while the corresponding figures for *M. sativa* (variety Provence) were July 4, 1950, and 43 days.

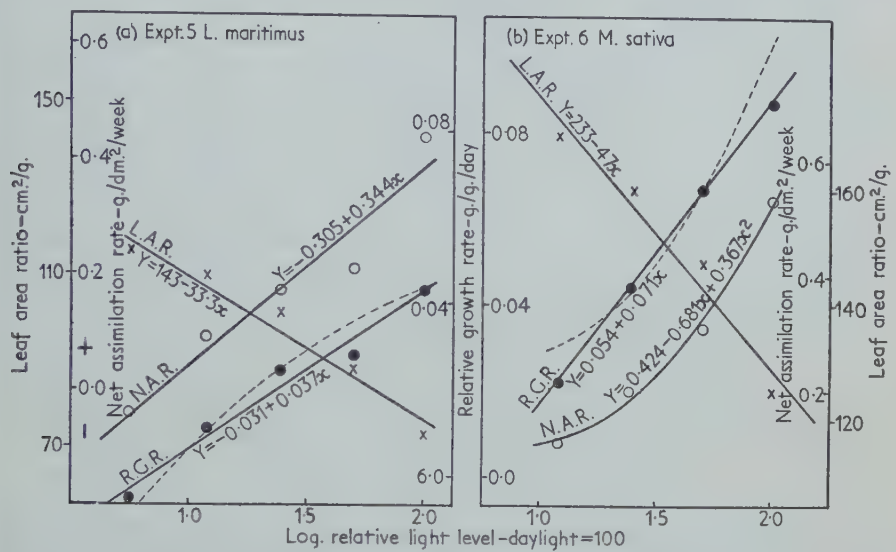


FIG. 3. The effects of varying light level on the net assimilation rate (NAR), leaf-area ratio (LAR) and relative growth-rate (RGR) of *Lathyrus maritimus* and *Medicago sativa*. The mean diurnal light received by the unshaded plants in experiments 5 and 6 was 24,853 and 27,373 foot-candle hours respectively.

The results of the two experiments are given in Fig. 3. For *M. sativa* the relationship between net assimilation rate and the logarithm of the light level is curvilinear while that for the leaf-area ratio is linear (the deviation is not significant). It is also to be noted that the derived equation does not fit the data for relative growth-rate as well as a fitted linear regression.

The reactions of *L. maritimus* to reduced light are somewhat different. In this instance for both net assimilation rate and leaf-area ratio the relationship on a logarithmic scale is linear. It is of interest that this linearity holds although the greatest degree of shading imposed on the plants is below the compensation point. Neither the derived curvilinear equation nor the fitted linear equation follow very closely the observed changes in the relative growth-rate.

The remaining three leguminous species which were examined in some detail consisted of *Trifolium hybridum*, *T. pratense* (Aberystwyth strain 151), and *T. repens*, of which a pure strain of the Ladino type was obtained from Dr. R. L. Lovorn of the United States Department of Agriculture. These

species were combined together in a single experiment so that direct comparisons could be made. The seed was sown on May 26, 1950, and the experiment started 54 days later. Because of the large number of pots the initial and final samplings were spread over 3 days.

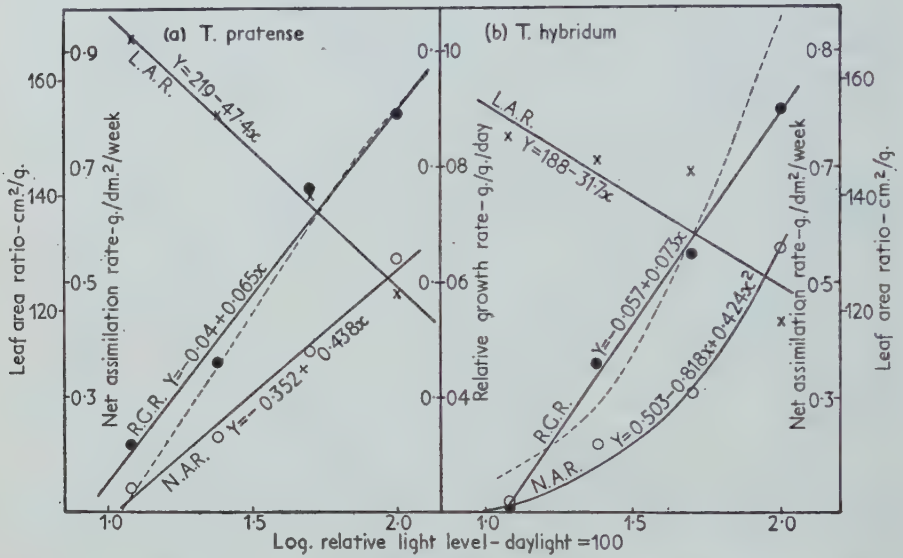


FIG. 4. Experiment 7. The effects of varying light level on the net assimilation rate (NAR), leaf-area ratio (LAR) and relative growth-rate (RGR) of *Trifolium pratense* and *T. hybridum*. The mean diurnal light received by the unshaded plants was 33,385 foot-candle hours.

For *T. pratense* the changes in both the net assimilation rate and the leaf-area ratio induced by shading follow logarithmic trends (Fig. 4*a*). It is not therefore surprising that the curvilinear equation derived from the two linear regressions is in reasonable agreement with the observed reductions in the relative growth-rate. The degree of curvilinearity is small and the directly calculated regression of closest fit is linear.

The reactions of *T. hybridum* to the same experimental conditions are somewhat different—see Fig. 4*b*. The relationship between net assimilation rate and the logarithm of the light received is not linear but curvilinear. As with *T. pratense* the leaf area ratio responds in a logarithmic manner but the fit to the data is indifferent. On the other hand, the derived equation for the changes in the relative growth-rate is reasonably accurate; nevertheless the fitted regression is again linear.

With the third species *T. repens* the pattern is once more divergent (Fig. 5*a*). For both net assimilation rate and leaf-area ratio, there is a curvilinear relationship with the logarithm of the light level. On the basis of these two equations it would be predicted that the changes in the relative growth-rate would follow a sigmoidal curve whereas in fact the points fall on a linear regression with the logarithm of the mean daily radiation.

A second experiment on *T. repens* was carried out in the following year when the seed was sown on June 11 and the experiment started on July 24. The degree of shading was also extended to include 0·055 daylight. A comparison of Fig. 5*a* and *b* shows that the trends differ in some respects. When the degree of shading is plotted logarithmically the fall in the net assimilation

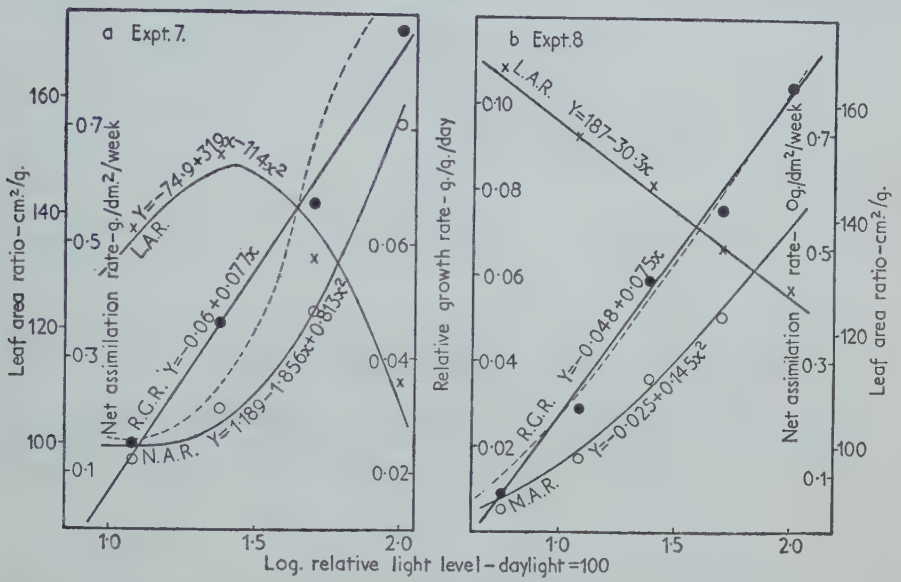


FIG. 5. *Trifolium repens*. The effects of varying light level on the net assimilation rate (NAR), leaf-area ratio (LAR) and relative growth-rate (RGR). The mean diurnal light received by the unshaded plants in experiments 7 and 8 was 33,385 and 32,269 foot-candle hours respectively.

rate for the second experiment is also curvilinear but the regression for leaf-area ratio is linear. For the changes in the relative growth-rate there is little to choose between the predicted equation and the fitted linear regression.

A further somewhat simpler experiment was carried out on two other legumes, *Vicia faba* and *Phaseolus multiflorus*. Although there were only three light treatments the data of Fig. 6 show marked differences in the reactions of the two species. The changes in the net assimilation rate and leaf-area ratio would seem to follow logarithmic trends in the case of *V. faba* while they clearly do not do so for *P. multiflorus*.

3. *The influence of shading on the growth of some grasses*

Altogether five species were examined in the early vegetative phase, that is each experiment was started some 31 to 47 days after the pots were sown. For three species, *Dactylis glomerata*, *Phleum pratense*, and *Lolium perenne* a simultaneous comparison was made of strains representing 'hay' and 'grazing' types produced by the Welsh Plant Breeding Station. The individual experiments lasted either 12–15 days (*D. glomerata*, *Lolium multiflorum*, *Festuca pratensis*) or 20–21 days for the two remaining species (*P. pratense*, *L. perenne*).

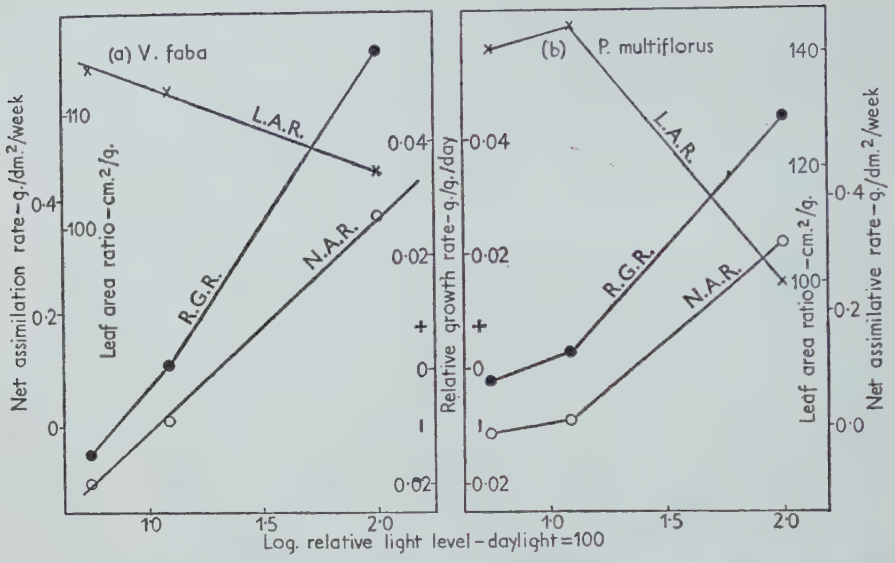


FIG. 6. Experiment 9. The effects of varying light level on the net assimilation rate (NAR), leaf-area ratio (LAR) and relative growth-rate (RGR) of *Vicia faba* and *Phaseolus multiflorus*. The mean diurnal light received by the unshaded plants was 29,511 foot-candle hours.

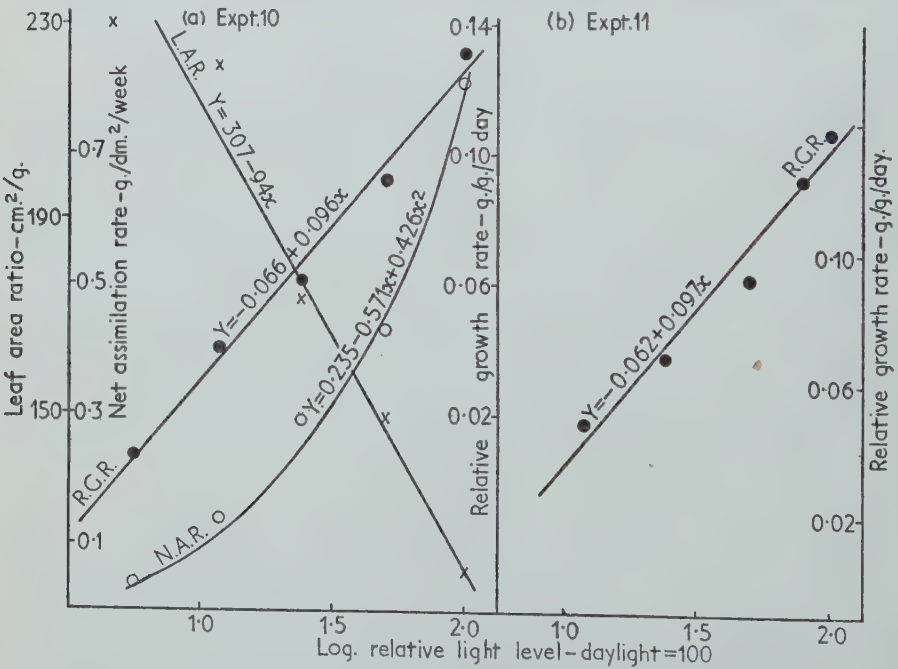


FIG. 7. *Lolium multiflorum*. The effects of varying light level on the net assimilation rate (NAR), leaf-area ratio (LAR) and relative growth-rate (RGR). The mean diurnal light received by the unshaded plants in experiments 10 and 11 was 35,346 and 28,310 foot-candle hours respectively.

Although the leaf laminae were separated from the rest of the shoot, only in one experiment on *L. multiflorum* were the areas measured. The reasons for this were twofold. In the first place, it is difficult to obtain valid estimates of the operative area when the laminae are not flat. Secondly, the green leaf sheaths and stems made up a considerable proportion of the total assimilating surface and since it was not possible to assess this contribution considerable errors would have been introduced in calculating the net assimilation rate.

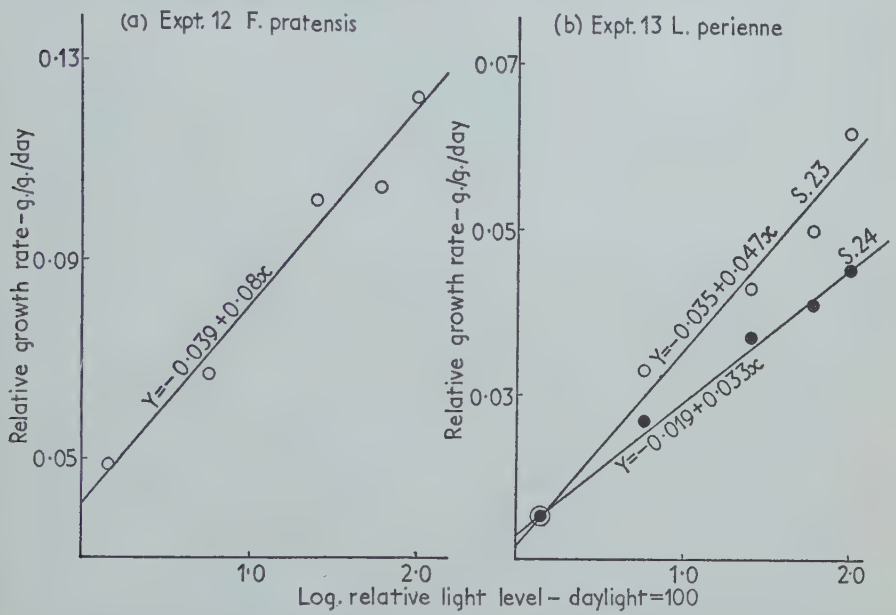


FIG. 8. The effects of varying light level on the relative growth-rate of *Festuca pratensis* and *Lolium perenne*. The mean diurnal light received by the unshaded plants in experiments 12 and 13 was 33,855 and 26,551 foot-candle hours respectively.

Accordingly for these species only the relative growth-rates will be considered: the changes in leaf weight and leaf-weight ratio have been discussed elsewhere (Blackman, 1956).

The reactions of *L. multiflorum* to shading are shown in Fig. 7. In both experiments, the relationship between relative growth-rate and the logarithm of the light level is best satisfied by a linear regression. In the experiment where the net assimilation rate was determined, the reduction in rate follows a curvilinear relationship with the logarithm of daily radiation while the corresponding but inverse relationship for leaf-area ratio is linear.

For *F. pratensis* the relative growth-rate is logarithmically related to the level of shading (Fig. 8*a*). Similar trends are also exhibited by both the strains of *L. perenne* (Fig. 8*b*). It is to be noted that S. 23 has on average a significantly higher growth rate than S. 24.

The results for the remaining two species are given in Fig. 9*a* and *b*. For *P. pratensis* the relative growth-rate of S. 48 is directly related to the logarithm

of the light level but for S. 50 the relative growth-rate is curvilinear. There is a similar divergency in the reaction of the two *D. glomerata* selections S. 37 and S. 143. It may be of some ecological significance that in both species it is the 'hay' strains (S. 48 and S. 37) which are more light demanding: on the other hand this is not so for *L. perenne*.

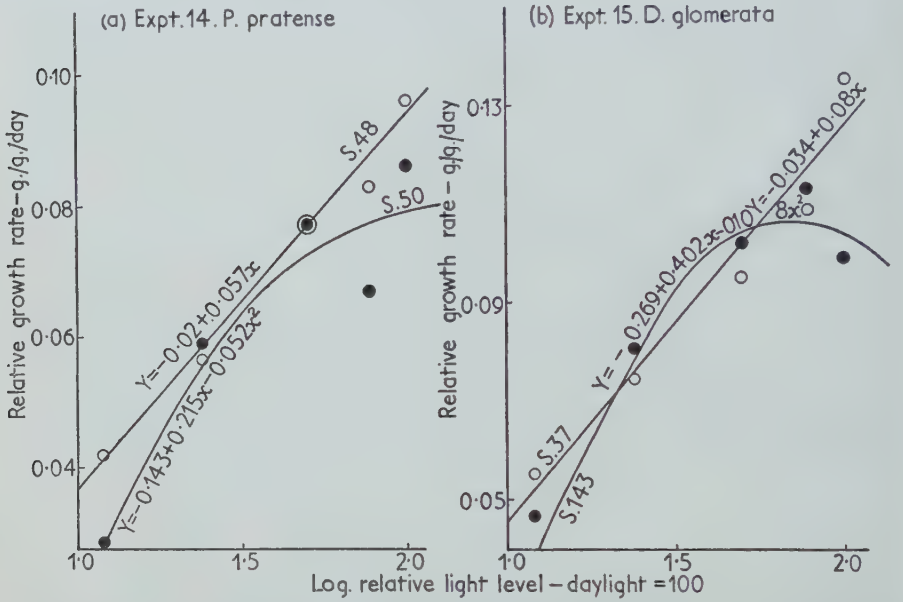


FIG. 9. The effects of varying light level on the relative growth-rate of two strains of *Phleum pratense* and *Dactylis glomerata*. The mean diurnal light received by the unshaded plants in experiments 14 and 15 was 25,998 and 36,225 foot-candle hours respectively.

DISCUSSION

The results of this investigation fully confirm previous conclusions as to the ways in which the light factor operates in controlling growth and development in the vegetative phase. For each of the new species examined halving the daily radiation received substantially reduces the net assimilation rate. From these facts and from the general trends of the curves of Figs. 1–7 it can be concluded that during the season of active growth the quantity of light reaching the individual plants was limiting the rate of photosynthesis. This quantity varied between experiments due to seasonal effects, but nevertheless in a number of experiments lay within the range of 32,000–37,000 foot-candle hours, that is during the hours of daylight the light intensity averaged 1,900–2,200 foot-candles.

In the earlier investigations (Blackman and Rutter, 1948, and Blackman and Wilson, 1951*a*) it had been found that a logarithmic relationship best expressed the changes in the net assimilation rate induced by shading. However, the present results have demonstrated that when the degree of shading

is extended to 0·055 daylight a logarithmic relationship may no longer hold for all species and all conditions. In fact for only *L. maritimus* and *T. pratense* (Figs. 3 and 4) is the relationship best satisfied by a logarithmic regression; in the remainder the relationships although curvilinear are not logarithmic and this departure is also consistent in the four sunflower experiments. When the data of Fig. 6 are taken into consideration it would seem that if the level of light ranges from below the compensation point to full daylight then for some species the pattern of change in the net assimilation may be sigmoid because a shift in level in the neighbourhood of the compensation point may cause little variation in the assimilation rate, e.g. *P. multiflorus*. Clearly this sigmoidal response will have ecological significance since such species will be better able to survive periods of intense shading than those in which the response is, for example, logarithmic. In this respect, *V. faba* and *L. maritimus* would be classed as intolerant of deep shade.

Turning to the variation in the leaf-area ratio induced by shading it has already been pointed out that the general trends and the major specific differences have been discussed in a previous paper (Blackman, 1956). It is sufficient therefore to comment that when the degree of shading is extended below 0·1 daylight the ratio may no longer continue to rise, especially if the mean radiation received by the unshaded plants is low. For example, this change is most pronounced in the first of the *H. annuus* experiments which was carried out in the short days of autumn—Fig. 1.

In the present experimental procedure the plants were raised in full daylight before they were shaded, but nevertheless it would be expected that the curve linking the ratio with the level of light must have a point of inflexion, since when *H. annuus* is subjected continuously to very low intensities the leaves fail to expand and as a result the leaf-area ratio is very small. Williams (1956) has pointed out that species react very differently in their leaf expansion to conditions inducing etiolation and this in turn will influence the position of the point of inflexion. Another complicating factor in comparing the experiments either within or between species is the positive influence of temperature on the ratio (Blackman, 1956). By and large it can be concluded that as long as the level of shading is not reduced below 0·2 daylight during the summer months then the change in the ratio follows an approximate logarithmic pattern for all species.

The previous studies demonstrated that even slight shading brings about a depression in the growth-rate of a number of species and this has also been found for all the five legumes examined, together with *L. multiflorum*, *L. perenne*, and *F. pratensis*. Indeed, the general implication is that in most instances the growth-rate of the unshaded plants was limited by the daily light energy received during the experiment.

When direct comparisons can be made between species, as is the case for the three species of *Trifolium*, reductions in the growth-rate caused by shading were similar. On the other hand, it is clear that for the grasses the light response can be determined by the strain. With *D. glomerata* S. 37 was more

intolerant of slight shade than S. 143 (Fig. 96), while for *P. pratense* there was a similar diversity between S. 50 and S. 48 (Fig. 9*a*). It is also to be noted that the superior growth-rate of the S. 23 strain of *L. perenne* was maintained at all except the deepest level of shade (Fig. 8*b*).

Since the relative growth-rate is an estimate of the mean response over the experimental period, errors of interpretation may be introduced if the process of adaptation to the different shade treatments is slow. The errors are likely to be greatest when the experimental period is short, the degree of shading imposed is large and the plants make little growth. In the individual experiments the plants were subjected to shading for at least 12–14 days and in the majority for 20–21 days. During the experimental period, save for *L. perenne* and *L. maritimus*, the gain in weight with 0·5 daylight was 3–6 fold and 2–3 fold for 0·24 daylight. On these grounds it would seem that conclusions about the high light requirements of the faster-growing species will not be subject to much uncertainty. Blackman and Wilson (1951*b*) have pointed out that the optimal light level for growth can be predicted from the regressions for net assimilation rate and leaf-area ratio, if these are approximately linear. They also emphasized that the errors of prediction will be minimal if the data for the leaf-area ratios are those for the final sampling occasion, since by then the plants are most likely to have reached a phase of equilibrium with the individual light treatments. Calculations along these lines have been made and with a view to minimizing the errors due to a non-linearity of the logarithmic regressions only the data for 0·24–1·0 daylight have been used. The results are seen in Table 1. For *M. sativa* and *T. hybridum* the high light requirements are confirmed: for *T. repens* the somewhat conflicting results may be due to the possibility that in the 1950 experiment some self-shading may have occurred at the end of the period. For the remainder this treatment of the data would suggest that the optimal light level is either full daylight or a little below it.

TABLE 1

Calculated Light Levels for the Maximal Relative Growth-Rate of Different Species

Number of expt.	Species	Optimal light level (daylight = 1·0)
5	Medicago sativa	2·51
7	Trifolium hybridum	1·48
	,, pratense	1·00
	,, repens	0·85
8	,, ,,	1·85
6	Lathyrus maritimus	0·76
9	Lolium multiflorum	0·71
1	Helianthus annuus	1·14
2	,, ,,	1·15
3	,, ,,	0·87
4	,, ,,	1·14

Included in Table 1 is a similar analysis of the four *H. annuus* experiments and it would seem that in three out of the four experiments the radiation received by the unshaded plants was below the optimal. It is known that the adaptation of *H. annuus* to a change in light intensity is rapid (Blackman and Wilson, 1954), and since in each experiment the relationship between shading and the relative growth-rate is logarithmic (Figs. 1 and 2), this provides further confirmatory evidence that the natural daylight was sub-optimal.

Since one of us has recently reviewed the work of other investigators bearing on the influence of the light factor on the growth of herbage plants (Black, 1957) it is sufficient to comment that even a moderate level of shading has caused a reduction in growth or dry-matter production of many grassland species in diverse parts of the world. Thus, it would seem that when other environmental factors are favourable growth in many regions may be limited by the diurnal radiation. The conditions when this limitation is likely to apply will be discussed in a subsequent paper.

Literature Cited

Black, J. N., 1957: The Influence of Varying Light Intensity on the Growth of Herbage Plants. Herb. Abstr., **27**, 89.

Blackman, G. E., 1956: Influence of Light and Temperature on Leaf Growth in 'The Growth of Leaves', ed. F. L. Milthorpe. Butterworth, London.

—— Black, J. N., and Kemp, A. W., 1955: Physiological and Ecological Studies in the Analysis of Plant Environment. X. An Analysis of the Effects of Seasonal Variation in Daylight and Temperature on the Growth of *Helianthus annuus* in the Vegetative Phase. Ann. Bot., N.S., **19**, 527.

—— —— and Martin, R. P., 1953: Physiological and Ecological Studies in the Analysis of the Plant Environment. VIII. An Inexpensive Integrating Recorder for the Measurement of Daylight. Ibid. **17**, 529.

—— and Rutter, A. J., 1948: Physiological and Ecological Studies in the Analysis of Plant Environment. III. The Interaction between Light Intensity and Mineral Nutrient Supply in Leaf Development and in the Net Assimilation Rate of the Bluebell (*Scilla non-scripta*). Ibid. **12**, 1.

—— and Wilson, G. L., 1951*a*: Physiological and Ecological Studies in the Analysis of Plant Environment. VI. The Constancy for Different Species of a Logarithmic Relationship between Net Assimilation Rate and Light Intensity and its Ecological Significance. Ibid. **15**, 63.

—— —— 1951*b*: Physiological and Ecological Studies in the Analysis of Plant Environment. VII. An Analysis of the Differential Effects of Light Intensity on the Net Assimilation Rate, Leaf-area Ratio, and Relative Growth Rate of Different Species. Ibid. 373.

—— —— 1954: Physiological and Ecological Studies in the Analysis of Plant Environment. IX. Adaptive Changes in the Vegetative Growth and Development of *Helianthus annuus* induced by an Alteration in Light Level. Ibid. **18**, 71.

Williams, W. T., 1956: Etiolation Phenomena and Leaf Expansion in 'The Growth of Leaves', ed. F. L. Milthorpe. Butterworth, London.

Protein Composition in Relation to Age of Daffodil Leaves

BY

MARY BRYANT[1] AND L. FOWDEN

(*Department of Botany, University College, Gower St., London, W.C.* 1)

Abstract

Daffodil foliage leaves were divided into sections along their length; the basal sections then contained the youngest, growing regions of the leaves, and the other sections represented progressively older tissue as the leaf apex was approached. Representative protein fractions were isolated from some of these sections, and after hydrolysis their amino-acid compositions were compared. Protein from bulb scale leaves was also analysed. Within the foliage leaf, age did not markedly affect the composition of the proteins. Larger differences of composition were found when the proteins of the bulb scale, a typical storage tissue, were compared with those of the foliage leaves.

The free amino-acid complements of the different sections of the foliage leaves were also compared. Variation of composition with leaf age did occur, but no generalizations can be made that are applicable to all amino-acids.

Introduction

DETAILED studies of the nature and composition of plant proteins were first made by Osborne (1924). His most important contributions concerned the proteins of storage organs, particularly seeds, and he noted that considerable differences existed in the protein compositions of various seed types. These differences tended to be greatest when the proteins of monocotyledonous seeds were compared with those of dicotyledonous seeds, and were reflected in the amino-acid composition of the whole or 'bulk' protein as well as in the relative amounts of the four main protein types (globulin, albumin, glutelins, and gliadins) making up the bulk protein of the seeds. Many recent investigations have served to confirm the validity of these earlier conclusions and have extended our exact knowledge of the amino-acid composition of seed proteins. Monocotyledonous seeds are now known to store proteins mainly of the glutelin and gliadin types and so, like glutelins and gliadins themselves, the bulk proteins contain large amounts of glutamic acid, proline, and amide-nitrogen but little lysine, histidine, and arginine. Dicotyledonous seeds contain primarily globulins and albumins (proteins markedly different in their amino-acid composition from the glutelins and gliadins) and so their bulk proteins are richer in their basic amino-acid content and do not contain glutamic acid, proline, and amide-nitrogen in excess.

Later workers also extended the scope of Osborne's studies to include the proteins from many other plant tissues. Globulins and albumins are widely

[1] Present address: Institute for Plant Physiology, Rothamsted Experimental Station, Harpenden, Herts.

[Annals of Botany, N.S. Vol. 23, No. 89, 1959.]

distributed in the vegetative parts of plants, and generally constitute the major proportion of the bulk protein of mature tissue. An approximate constancy of amino-acid composition is then to be expected for the proteins of such 'active' organs. The bulk protein compositions obtained for barley leaves (Yemm and Folkes, 1953), legume leaves (Tristram, 1949), *Chlorella* (Fowden, 1951*a*), and yeast (Lindan and Work, 1951) are indeed strikingly similar and lend support to this generalization. However, this constancy of composition does not extend to the proteins of storage organs, and the compositions of potato tuber, tulip bulb, and carrot-root proteins have been shown to differ considerably from those of the above active tissues (Steward and Thompson, 1954).

This constancy of bulk protein composition determined for tissues of different species does not necessarily indicate that a comparable mixture of individual protein molecules is present in all the tissues; different mixtures of individual proteins may exist and yet possess *mean* amino-acid compositions that are approximately constant. There is evidence that during the development of a particular tissue or organ the balance of metabolism can change, and that this is associated with changes in the concentrations of individual proteins or enzymes present in the tissue (Robinson and Brown, 1954). It would be instructive therefore to study the bulk protein composition of a particular tissue during its ageing in order to ascertain to what extent a changed metabolism may reflect itself in the mean protein composition of the tissue.

Daffodil leaves provide a suitable material for this type of study and have been used in the present investigation. These leaves grow from a basal meristem, so that serial sections taken from the base to the apex of a leaf represent tissue of gradually increasing physiological age (Pearsall and Billimoria, 1937). Sections taken in this way also show a progressively altered metabolism. The extreme basal section of the leaf is white and incapable of effecting photosynthesis. Photosynthesis may also be limited in the apical section, which often appears senescent. The sections differ also in features of nitrogen metabolism (Pearsall and Billimoria, 1937, 1938, 1939). Presumably these variations in metabolic activity can be ascribed to an altered balance of enzyme concentrations within the different sections of the leaf. The daffodil leaves may therefore be employed satisfactorily to determine the extent to which changes in the physiological behaviour of a plant tissue are reflected in its overall protein composition.

The bulbs provide scale leaves, which can be regarded as storage tissue and whose metabolic activity is very low when compared with the normal foliage leaf. If differences in the amino-acid composition of the leaf proteins exist, they might be expected to be most marked when bulb scale and foliage leaves are compared.

The free amino-acids present in the leaves have also been compared.

The results on daffodil leaves can be compared with those obtained in two previous investigations. In the first of these the complete amino-acid composition of the bulk proteins of samples of *Chlorella* cells of different physiological

age were determined (Fowden, 1952), whilst in the second the protein composition of lupin leaves were assayed from the primordial to the fully-expanded stage of development (Steward, Whetmore, Thompson, and Nitsch, 1954).

Experimental

Plant material: Daffodils (var. King Alfred) were used in all experiments, and were grown under uniform conditions. Leaves were harvested at three different stages of plant development. In the form of bulb scales, they were collected from 20 plants in January at the time when the foliage leaves were just beginning to emerge from the bulbs. Foliage leaves were collected from 40 plants when the flower buds first became apparent. These leaves, which were not yet fully expanded, were divided into five sections of equal length. The basal sections consisted entirely of white, chlorophyll-free cells, and contained the leaf meristem. The next sections were of a yellowish-green colour and were from a region of the leaf that was expanding largely by a process of cell elongation. The third, fourth, and apical sections were green, mature regions of the leaf. A further collection of foliage leaves was made at the time of full-flowering of the plants. These leaves, now fully grown, were divided in the same way as the previous batch into five sections of equal length.

Each of the tissue samples so obtained was further divided for analysis as follows. A representative portion was dried at 95° for 45 min. and then at 60° to constant weight. The dried residue was then ground to a fine uniform powder and used for the subsequent determinations of the free amino-acids in the tissue. No hydrolysis of protein took place during this drying procedure since the protein-N to non-protein-N ratio remained unaltered. Another portion was extracted immediately, or was stored at −15° until extracted, to yield a purified protein preparation as described below. A third, smaller portion was used to obtain dry weight and total nitrogen values.

Leaf protein extraction: The method of protein extraction was based on that used for *Chlorella* proteins (Fowden, 1951*a*). The leaf tissue was macerated and exhaustively extracted with pH 9·0 borate buffer; the extracted proteins were then flocculated by adjusting the extract to pH 4·5.

Fresh leaf tissue was macerated in an Atomix blendor (Measuring and Scientific Equipment Ltd.) with about twice the amount of 0·2 M-sodium borate buffer at pH 9·0, and a little *n*-octanol to prevent frothing. The pH of the macerate, now 8·6, was readjusted to pH 9·0 by addition of N-KOH. The grinding was continued in an all-glass wet homogenizer, after which the cell debris was removed by centrifugation. The extract was decanted and the debris was re-extracted in more borate buffer. The resulting residue was extracted twice more. On adjusting to pH 4·5, protein was precipitated from the first three fractions but not from the fourth, so indicating that almost complete extraction had been achieved. The precipitated protein was coagulated by warming to 70° for 10 min., and removed by centrifugation. The

precipitated protein was then washed twice with dilute acetic acid, pH 4·5, six times with boiling ethanol, and finally twice with hot ether. Little pigment adhered to the protein after this treatment, and after drying at room temperature it was ground finely to give a light buff-coloured powder.

Nitrogen determinations indicated that the proteins extracted represented 90 per cent. or more of the total protein originally present in the tissues. The protein fractions can then be considered to be entirely representative of the whole or bulk protein of the leaves.

Protein hydrolysis: This was carried out using a mixture of equal volumes of glacial acetic acid and conc. A.R.HCl containing 1 per cent. (w./v.) $SnCl_2.2H_2O$. One ml. of this acid mixture was used for each 3 mg. of protein sample. The hydrolyses were carried out in sealed ampoules heated at 100° for 24 hr. At the end of this time the hydrolysis mixtures remained a light amber colour; no humin formation occurred. The hydrolysates were then diluted and evaporated to dryness *in vacuo*. The residues were redissolved in water and repeatedly evaporated *in vacuo* over solid NaOH to remove the last traces of free acid. The final residue was dissolved in water to give a solution containing 25–50 μg. α-NH_2-N per 100 μl., which could be applied directly to paper chromatograms.

Amino-acid estimations: These were made after the protein hydrolysates had been resolved into their component amino-acids by means of one- or two-dimensional paper chromatograms following methods essentially the same as those of Fowden (1951*b*, 1954). 100 μl. of each protein hydrolysate solution were used in the preparation of a chromatogram. For two-dimensional chromatography, Whatman no. 3MM filter-paper sheets (24 in. $\times$ 24 in.) were used, 75 per cent. (w./w.) phenol being used as the first developing solvent and an *n*-butanol-acetic acid mixture (Partridge, 1948) as the second. The following amino-acids were conveniently separated on one-dimensional butanol-acetic acid chromatograms: tyrosine, valine and methionine as one spot, phenylalanine, and the leucines after 30 hr. development, and arginine, histidine, and lysine after 9 days' development. Location and estimation of the separated amino-acids followed the method of Fowden (1954), with the following modifications. The ninhydrin reagent of Moore and Stein (1948) was replaced by that of Yemm and Cocking (1955). All determinations were made in triplicate and final values were based upon determinations of known mixtures of amino-acids separated and estimated simultaneously with the hydrolysis mixtures. The final values obtained were subject to an error of about ± 5 per cent.

Free amino-acids present in the dried and powdered leaf samples were extracted by shaking with cold 80 per cent. (v./v.) ethanol (1 ml. for each 10 mg. dried leaf material) for 24 hr. The residue was re-extracted three more times. No nitrogen-containing compounds were present in the fourth extract, so the first three were combined, and the pooled extract was evaporated to dryness *in vacuo* below 60°. The residue was redissolved in water and measured small amounts were used for the determination of total soluble-N. The

remainder was slowly run through a column packed with Zeocarb 215, mesh 80 (Permutit Co. Ltd.) in the H^+ form. The column was then washed free of sugars, organic acids, and other ethanol-soluble, non-cationic substances. The absorbed amino-acids were finally eluted quantitatively from the column using 3N-NH_3. The eluate was evaporated to dryness and the residue was redissolved in a suitable small volume of water to give a solution containing

Table 1

Shows the Results of the Fractionation of the Nitrogenous Compounds of Daffodil Leaves of Different Ages into Soluble and Insoluble Components

(Data expressed as g. N per 100 g. dry weight of tissue)

Tissue	Total nitrogen	70% ethanol soluble non-protein nitrogen	70% ethanol insoluble protein nitrogen	Soluble nitrogen as % of total N.
January collection:				
Bulb scales	1·14	0·46	0·68	40·3
Before flowering stage:				
Foliage leaves, basal section .	3·40	0·51	2·89	15·0
Foliage leaves, third section .	3·48	0·60	2·88	17·2
Foliage leaves, apical section .	3·26	0·55	2·71	16·9
Full-flowering stage:				
Foliage leaves, basal section .	2·52	0·60	1·92	23·8
Foliage leaves, third section .	2·94	0·64	2·30	21·8
Foliage leaves, apical section .	3·22	0·55	2·67	17·1

25–50 μg. α-NH_2-N per 100 μl., which was applied directly to paper chromatograms. Unless this step was used to remove non-cationic substances from the 80 per cent. ethanol extracts, serious streaking occurred on the developed paper chromatograms.

Quantitative determinations of the amino-acids present in these final solutions were made using paper chromatography as above.

Total N determinations were by micro-Kjeldahl digestion following the procedure of Chibnall, Rees, and Williams (1943).

Amide-N was determined as NH_3-N by nesslerization after preliminary hydrolysis with N-H_2SO_4 for 3 hr. at 100°.

Total α-NH_2-N determinations were made by the method of Yemm and Cocking (1955).

Tryptophan was determined by procedure K of Spies and Chambers (1949).

Results

The total nitrogen contents of the leaves used are recorded in Table 1, together with the results of the fractionation into 70 per cent. ethanol-soluble non-protein-nitrogen and insoluble protein-nitrogen components. The total nitrogen content of the bulb scale leaves is much less than that of the foliage leaves. This results from a large decrease of the protein nitrogen content

and not from any marked alteration in the level of the total non-protein-nitrogen compounds. When the non-protein nitrogen is expressed as a percentage of the total nitrogen of the leaves it will be seen that this percentage is far higher for the bulb scales than for normal foliage leaves. These observations are in agreement with the accepted idea that a transition from a dormant storage tissue to an actively metabolizing one is associated with an increase within the tissue of the ratio protein nitrogen/non-protein nitrogen.

TABLE 2

Shows the Amino-acid Compositions of the Protein Fractions Isolated from Daffodil Leaves of Differing Age, together with the Total Nitrogen Contents of the Isolated Protein Samples

(Data expressed as g. amino-acid N per 100 g. total protein nitrogen)

Amino-acid	Before flowering stage Foliage leaf			Full-flowering stage Foliage leaf			Bulb scale leaf
	Basal section	Third section	Apical section	Basal section	Third section	Apical section	
Aspartic acid	5·1	4·6	4·4	4·5	4·4	4·7	11·7
Glutamic acid	6·3	6·2	5·7	5·5	5·6	6·3	5·9
Serine	4·8	4·2	5·2	2·8	2·8	3·3	5·3
Glycine	5·6	4·7	6·3	5·5	5·6	5·4	8·8
Threonine	3·6	3·8	3·5	4·3	4·6	5·1	8·9
Alanine	5·3	4·9	5·2	5·1	4·9	5·7	6·9
Valine+methionine	4·1	4·7	4·8	5·5	5·3	5·8	3·4
Leucines	7·5	7·5	7·2	8·5	8·9	9·7	7·1
Phenylalanine	3·9	4·0	4·0	4·6	4·9	5·0	4·7
Tyrosine	3·0	3·8	4·0	4·5	3·5	3·4	1·6
Tryptophan	—	—	—	0·8	0·9	0·8	—
Arginine	16·4	19·0	18·1	19·6	19·6	16·8	11·8
Histidine	5·6	9·8	10·0	6·0	5·2	5·2	6·3
Lysine	12·0	7·0	7·5	9·6	10·2	8·2	7·4
Amide-N	2·2	2·5	3·0	4·2	4·4	3·8	—
N accounted for	85·4	87·1	88·9	91·0	90·8	89·2	89·8
% N in isolated protein sample	12·0	8·7	8·1	13·2	12·9	13·5	12·4

Foliage leaves analysed before flowering had approximately equal nitrogen contents throughout their whole lengths. At this stage the leaf was still capable of further expansion, and presumably still possessed an active basal meristem. Leaves collected during the period of full flowering were fully developed. The total nitrogen contents of the basal sections were now less than those determined for the green portions of the leaves. These sections would be no longer meristematic, and they also lacked the ability to effect photosynthesis. They may then provide a type of tissue whose level of metabolic activity is intermediate between those of active and storage tissue; this conclusion is in accord with the fact that the protein-nitrogen/non-protein-nitrogen ratio of the tissue is also intermediate between those values recorded for the green leaf and the bulb scales.

The amino-acid compositions of the protein fractions isolated from the different leaf sections are recorded in Table 2, together with the total nitrogen contents of these fractions. These proteins were hydrolysed using hot mineral acid without any formation of humin, and so destruction of amino-acids

would be very small. This conclusion is supported by the fact that, although no estimates were made of the proline and cyst(e)ine contents, about 90 per cent. of the total protein-nitrogen was accounted for in the amino-acid analyses of each fraction.

The amino-acid compositions of the proteins of different sections of the foliage leaves show a close similarity to one another. Many of the differences recorded for the contents of individual amino-acids in different sections of the

TABLE 3

Shows the Levels of the Free Amino-acids Present in Daffodil Leaves of Differing Age

(Data expressed as μg. α-NH_2-N present in each amino-acid per g. dry wt.)

	Before flowering stage Foliage leaf			Full-flowering stage Foliage leaf		
Amino-acid	Basal section	Third section	Apical section	Basal section	Third section	Apical section
Aspartic acid	103	59	41	77	76	47
Glutamic acid	65	35	32	37	40	43
Serine	188	202	140	68	48	28
Glycine	115	80	45	45	55	52
Threonine	75	81	tr	57	37	22
Alanine	246	253	295	114	251	252
Tyrosine	0	0	0	94	138	57
γ-Aminobutyric acid	207	204	128	66	165	169
Leucines	63	99	106	118	154	141
Valine+methionine	71	80	60	0	50	38
Phenylalanine	74	79	56	110	120	118
Asparagine	262	445	118	262	194	67
Glutamine	260	185	46	232	138	119
Arginine	116	136	81	68	87	14
Histidine	tr	tr	tr	tr	tr	tr
Lysine	220	314	56	37	31	tr
Dry wt./fresh wt. ratio	0·125	0·133	0·180	0·118	0·148	0·219

tr = trace amount too small to determine.

same leaf are barely significant after allowing for experimental errors. Differences tend to be larger when the proteins of the leaves collected during full flowering are compared with those harvested before flowering. For instance, the leucines and amide-nitrogen are always present in larger amounts in proteins from fully-grown leaves, whilst the serine contents of these leaves are less than those of expanding leaves.

Larger differences are found when the composition of the proteins of the bulb scales is compared with those of foliage leaf proteins. Aspartic acid levels are much increased in the bulb proteins and smaller increases are found in the glycine, alanine, and threonine contents. In contrast arginine and valine are present in smaller amounts in the bulb proteins.

The amounts of free amino-acids found in different sections of foliage leaves are shown in Table 3. Data are expressed on a dry-weight basis, but since the amounts of non-protein nitrogen did not differ greatly from section

to section (Table 1), the concentrations of the various amino-acids relative to one another would remain almost unchanged as a proportion of the total soluble nitrogen. It will be seen that there is a tendency for the levels of many amino-acids to decrease from leaf base to apex although there are numerous exceptions to this generalization, e.g. the concentrations of the leucines and alanine increase as the leaf apex is approached.

The basal sections of daffodil leaves are noticeably more succulent than the apical portions; this observation is supported by determinations of the dry weight/fresh weight ratios of the leaves, which increase from leaf base to apex (Table 3). When expressed on a fresh-weight basis, the concentrations of many free amino-acids would tend to be far more uniform along the whole length of the leaves.

Discussion

The main conclusion to be drawn from these analyses is that differences in the physiological and metabolic behaviour of daffodil foliage leaves of different ages, which must be accompanied by changes in the balance of enzyme concentrations, are not associated with any marked changes of the composition of the bulk proteins isolated from the leaves. Larger differences were encountered when comparison was made of foliage leaf protein with the proteins of the bulb scale leaves. Since the latter leaves are regarded as typical storage tissue, these differences are not unexpected. Similar findings occur in earlier comparisons of growing and semi-dormant tissues of the same plant, e.g. when the proteins of growing tissue cultures of potato tuber or carrot root are compared with those of the mature non-growing storage tissues (Steward and Thompson, 1954). Certain similarities of composition can be noted between the proteins of tulip and daffodil bulbs; both contain relatively large amounts of glycine and aspartic acid.

The results obtained for foliage leaves agree with previous findings for the bulk proteins isolated from *Chlorella* cells of different ages (Fowden, 1952) and those from lupin leaves analysed at different stages of development (Steward *et al.*, 1954). These latter workers found that there was a very small decrease in the content of the basic amino-acids of the proteins from mature leaves when compared with those from primordial leaves. This type of result supports the idea that in rapidly dividing tissues, nuclear proteins of the histone and protamine types, which possess high contents of basic amino-acids, are more abundant than in older tissues in which cell division is either relatively slow or no longer active. Confirmation of this finding regarding the basic amino-acids cannot be found in either the daffodil or *Chlorella* work, where the levels of the basic amino-acids remain nearly constant at all stages of development. The validity of associating high levels of basic amino-acids with high concentrations of histone and protamine, i.e. with the process of cell division, seems now to be in doubt, since the proteins of certain storage tissues, e.g. tulip bulbs, contain very large amounts of arginine, although little cell division is occurring in most parts of the bulb.

Alanine, γ-aminobutyric acid, asparagine, glutamine, serine, and lysine all represent major components of the free amino-acid fraction of the leaves of daffodil (see Table 3). This finding is similar to that observed for many other leaves.

The concentrations of individual amino-acids are often smaller in leaves harvested during full-flowering than in those collected earlier. This may indicate that some translocation of soluble nitrogen-containing compounds (amino-acids and amides) from leaves to flower head takes place during the growth of the inflorescence. Other conclusions are difficult to make, partly because it is uncertain whether a dry weight or fresh weight representation of amino-acid concentrations provides the better index. The data as expressed in Table 3 appear quite different if calculated on a fresh weight basis. This basis seems in some ways to be the more useful index: at least the calculated levels of amino-acid concentrations give a better representation of those existing within the living plant. Many individual amino-acid concentrations (aspartic and glutamic acids, serine, threonine, the amides and arginine), which decrease with increasing leaf age in Table 3, then become far more constant in different sections of a leaf, since the dry weight/fresh weight ratio is approximately doubled in passing from leaf base to apex.

Comparison of these results obtained for daffodil with those for the free amino-acid concentrations present in developing lupin leaves (Steward *et al.*, 1954) is not very profitable, mainly because the latter leaves contain enormous amounts of asparagine relative to the other free amino-acids. They then represent a somewhat abnormal situation, in which changes of concentration of other free amino-acids tend to be masked by the high asparagine levels.

Summary

1. Representative samples of the proteins present in daffodil leaves of varying age have been isolated. These samples represented over 90 per cent. of the total protein present in the leaves.

2. The amino-acid composition of the proteins from foliage leaves was closely similar irrespective of the age of the leaf from which they were isolated.

3. Larger differences of amino-acid composition are encountered when the protein isolated from the bulb scale leaves is compared with that of the foliage leaves.

4. The variations of concentration of the free amino-acids present within leaves of different ages have been studied. Concentrations of many amino-acids tend to decrease with increasing leaf age if represented on a dry-weight basis, but are generally more constant when reported on a fresh-weight basis.

This investigation was carried out whilst one of us (M. B.) held a Post-graduate Research Studentship of the University of London. It was also aided by a grant from the Rockefeller Foundation.

Literature Cited

Chibnall, A. C., Rees, M. W., and Williams, E. F., 1943: The Total Nitrogen Content of Egg Albumin and Other Proteins. Biochem. J., **37**, 354.

Fowden, L., 1951*a*: The Composition of the Bulk Proteins of *Chlorella*. Ibid., **50**, 355.

—— 1951*b*: The Quantitative Recovery and Colorimetric Estimation of Amino Acids separated by Paper Chromatography. Ibid., **48**, 327.

—— 1952: The Effect of Age on the Bulk Protein Composition of *Chlorella vulgaris*. Ibid., **52**, 310.

—— 1954: The Nitrogen Metabolism of Groundnut Plants: the Role of γ-Methyleneglutamine and γ-Methyleneglutamic Acid. Ann. Bot., N.S., **18**, 417.

Lindan, O., and Work, E., 1951: The Amino Acid Composition of Two Yeasts used to produce Massive Dietetic Liver Necrosis in Rats. Biochem. J., **48**, 337.

Moore, S., and Stein, W. H., 1948: Photometric Ninhydrin Method for Use in Chromatography of Amino Acids. J. biol. Chem., **176**, 367.

Osborne, T. B., 1924: Vegetable Proteins. 2nd edn. revised. New York: Longmans, Green & Co.

Partridge, S. M., 1948: Filter-paper Partition Chromatography of Sugars. Biochem. J., **42**, 238.

Pearsall, W. H., and Billimoria, M. C., 1937: Losses of Nitrogen from Green Plants. Ibid., **31**, 1743.

—— —— 1938: Effect of Age and Season on Protein Synthesis in Detached Leaves. Ann. Bot., N.S., **2**, 317.

—— —— 1939: Influence of Light on Nitrogen Metabolism in Detached Leaves. Ibid., **3**, 601.

Robinson, E., and Brown, R., 1954: Enzyme Changes in Relation to Cell Growth in Excised Root Tissues. J. expt. Bot., **5**, 71.

Spies, J. R., and Chambers, D. C., 1949: Chemical Determination of Tryptophan in Proteins. Anal. Chem., **21**, 1249.

Steward, F. C., and Thompson, J. F., 1954: Proteins and Protein Metabolism in Plants. In The Proteins, Vol. IIa, p. 513. Ed. by Neurath, H., and Bailey, K. New York: Academic Press Inc.

—— Whetmore, R. H., Thompson, J. F., and Nitsch, J. P., 1954: A Quantitative Chromatographic Study of Nitrogenous Components of Shoot Apices. Amer. J. Bot., **41**, 123.

Tristram, G. R., 1949: Amino Acid Composition of Purified Proteins. Adv. Prot. Chem., **5**, 84.

Yemm, E. W., and Cocking, E. C., 1955: The Determination of Amino Acids with Ninhydrin. The Analyst, **80**, 209.

—— and Folkes, B. F., 1953: The Amino Acids of Cytoplasmic and Chloroplastic Proteins of Barley. Biochem. J., **55**, 700.

Zacharius, R. M., Cathey, H. M., and Steward, F. C., 1957: Nitrogenous Compounds and Nitrogen Metabolism in the Liliaceae. III. Changes in the Soluble Nitrogen Compounds of the Tulip and their Relation to Flower Formation in the Bulb. Ann. Bot., N.S., **21**, 193.

Mineral Content of Plantations of *Pinus sylvestris* L.

BY

J. D. OVINGTON

(*The Nature Conservancy, Merlewood, Grange-over-Sands*)[1]

With five Figures in the Text

Contents

Abstract

Data are given of the percentage composition and total contents of Na, K, Ca, Mg, P, and N in trees forming an age series of plantations of *Pinus sylvestris*. From these data, calculations are made of the total nutrient uptake by the trees, both annually and for the whole rotation, the removal of nutrients in the crop, the immobilization of nutrients within the trees, and the return of nutrients to the soil by litter fall and unharvested material from selective thinning and cropping. Comparisons are made of the nutrient uptake by the trees and typical agricultural crops, and the results are considered in terms of the maintenance of soil fertility.

Introduction

MANY new forests have been established in Britain within the last decade and significant changes in the landscape will occur as these forests mature. Usually fast-growing tree species are planted, and since much of the land available for forestry has soil relatively poor in nutrients, there appears to be some danger of nutrient depletion of the mineral soil as a result of tree growth. Little is known of the magnitude of nutrient uptake by different woodlands, but it varies considerably according to the tree species that has been established (Ovington, 1957*a* and in press).

In a previous paper (Ovington, 1957*b*) data were given of the dry weights of individual trees and of all the trees in each plantation for an age series of nine plots of *Pinus sylvestris* sampled in 1954. Since dry-matter production

[1] Now at the Dept. of Botany, University of Minnesota, Minneapolis 14, U.S.A.

[Annals of Botany, N.S. Vol. 23, No. 89, 1959.]

by the pine stands alters as the trees mature, it seemed likely that there would be corresponding changes in the incorporation of nutrients into the tree crop. Detailed chemical analysis of the samples has been completed and this account is concerned with the uptake of nutrients by the trees. In a following paper an attempt will be made to show the relationship between the nutrient content of the trees and the circulation of nutrients within the forest ecosystem as a whole. All the plantations are located within a fairly level, compact area of the state forest of Thetford Chase so that there is probably little climatic variation over the sample area. The soils are typical, sandy Breck soils, the nutrient content of which varies to some extent locally.

Methods

The method of selecting and sampling representative trees has been described (Ovington, 1957*b*). After drying the samples of the different parts of the trees, the samples were ground down so that they would pass through a sieve having holes of 0·4 mm. diameter. Part of the ground material was then digested with a mixture of nitric, perchloric, and sulphuric acids (Piper, 1950) and various elements in the resultant acid solution were determined. Sodium and potassium were measured with a flame photometer, calcium by the versenate technique, magnesium by the titan yellow method, and phosphorus by developing the molybdenum blue colour. The nitrogen content of the ground material was determined, using the micro-Kjeldahl technique.

By combining the dry-weight data with the results of the chemical analysis, it has been possible to estimate the total nutrient content of the individual trees and plantations as well as the annual uptake of nutrients at different stages of growth.

Results

1. *Percentage composition*

The chemical composition of the different parts of the individual trees varies considerably and in Fig. 1 the average amounts of different elements, as mg./100 g., are given for the whole of the leaves, cones, branches, boles, and roots of the sample trees. When the components of the trees are arranged in order of decreasing percentages of plant nutrients, the general order becomes leaves, branches, roots, cones, and boles, but some exceptions occur for specific elements. For example, the cones are relatively rich in potassium and phosphorus but poor in calcium, whilst some roots are rich in sodium. The nutrient content of the tree roots may be influenced to some extent by soil particles incorporated in the root tissue.

As the trees become older there is little evidence of a progressive change in the proportions of each of the six elements, except that the average values for potassium, calcium, magnesium, phosphorus, and nitrogen of the tree boles, and to a lesser extent of the branches, tend to decrease with age.

The nutrient status of the mineral soils of the different plots varies and, in order to see how far this affected the nutrient content of the trees, two soil

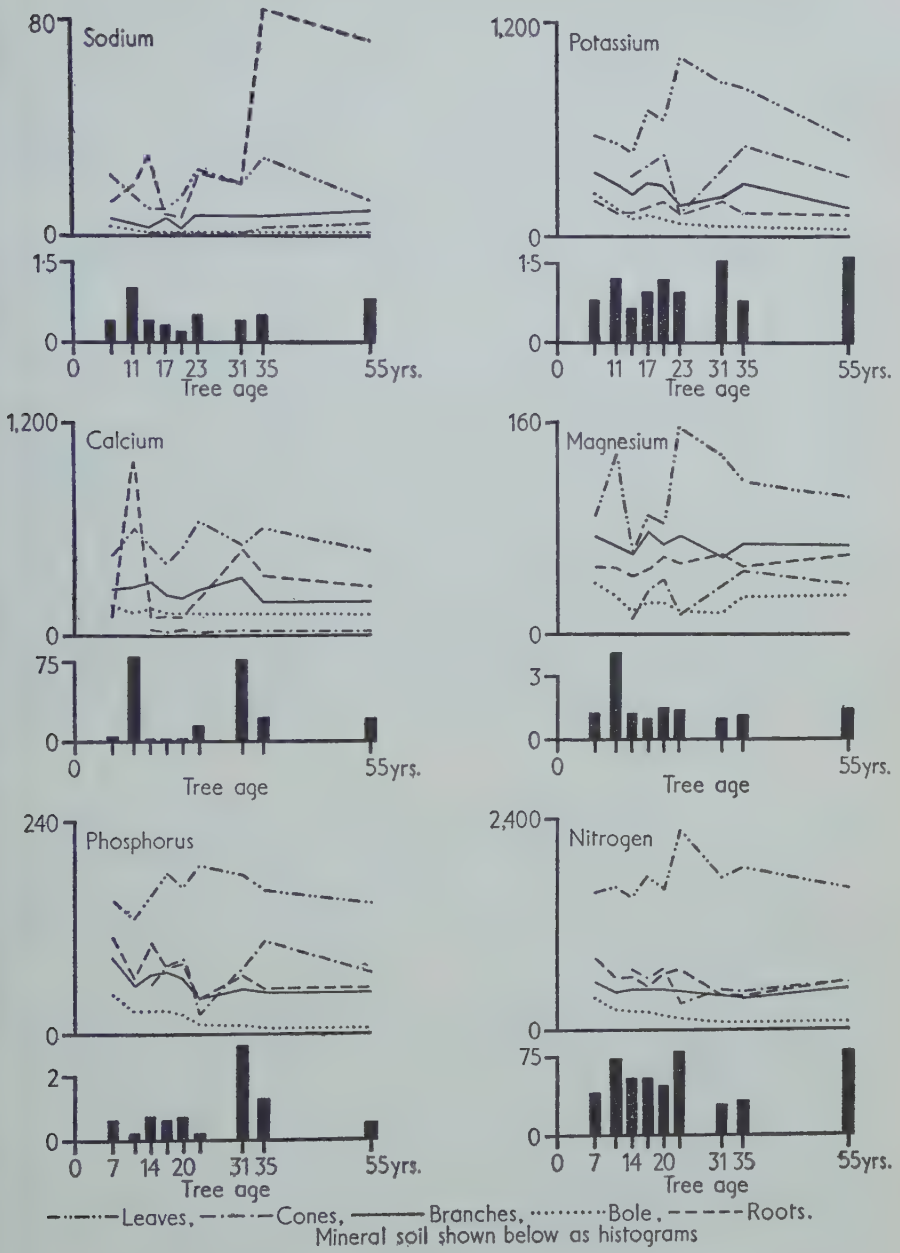


FIG. 1. The composition (mg./100 g. O.D.) of the components of the trees and mineral soil.

profiles in each plot were sampled to a depth of 50 cm., and the soils analysed. Total soil nitrogen was determined by the micro-Kjeldahl method and the exchangeable values of the other elements were determined using 2·5 per cent.

acetic acid as the extracting solution and the same analytical techniques as for the plant material. There is practically no correlation between variations in the nutrient status of the mineral soils of the plots and the chemical composition of the trees. Occasionally, some changes in the nutrient content of the trees, particularly of leaves, seem to reflect soil differences, e.g. the large proportion of calcium in the leaves of the eleven-year-old trees is associated with a high value of exchangeable calcium in the soil.

The results given in Fig. 1 are averages for the parts of the trees as a whole, but considerable variation of nutrient content may occur within each component (Fig. 2). Thus, the proportions of ash, sodium, and calcium in the leaves increase as the leaves become older, whilst potassium, phosphorus, and nitrogen decrease and magnesium shows no consistent difference. Changes in the nutrient content of the branches are less clearly defined than for the leaves, but there is a general tendency for the percentage nutrient content to decrease with age although a temporary build-up of calcium may occur in the second-year branches. The percentages of ash, phosphorus, magnesium, potassium, and nitrogen increase with height up the tree boles, but there is no corresponding increase in calcium or sodium which are more evenly distributed along the bole length and may even increase in amount at the base of the trunk. The fine rootlets have a very different chemical composition from the large lateral roots, containing greater percentages of ash, phosphorus, and nitrogen but smaller percentages of potassium and magnesium than the roots. There is no significant difference between the two root classes in their percentages of sodium and calcium.

2. *Nutrient content per tree*

As the trees become older and heavier, they contain greater amounts of plant nutrients, and the most rapid build-up of nutrients occurs from twenty years onwards (Fig. 3). The increase of nutrient content with greater tree age is closely associated with the increase in dry weight but is also affected by percentage composition.

The relative distribution of the various nutrients within the trees differs considerably as a result of the differences in dry weight and in the percentage composition of the component parts of the trees. Thus, for the pines of 35 to 55 years of age, the bole represents about 63 per cent. of the total dry weight but only contains 10 per cent. of the sodium, 28 per cent. of the potassium, 37 per cent. of the calcium, 37 per cent. of the magnesium, 16 per cent. of the phosphorus, and 19 per cent. of the nitrogen in the tree. Consequently, the harvesting of the tree boles represents a greater proportionate loss from the plantation of total calcium and magnesium than any of the other elements that have been determined.

3. *Nutrient content of the stock*

The number of trees per hectare decreases as the plantations mature because of the suppression of weaker trees and the gradual harvesting of the crop. Due

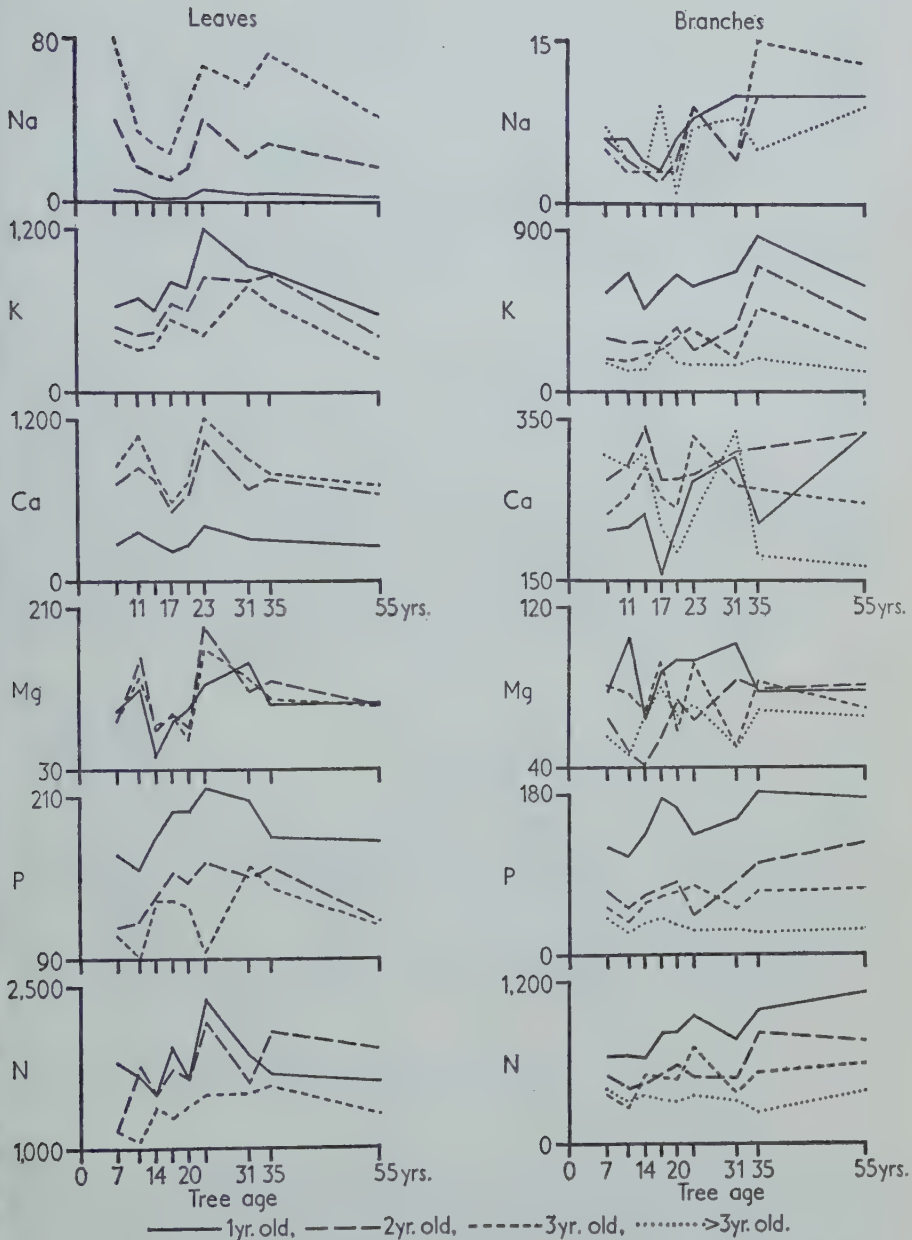


FIG. 2. The composition (mg./100 g. O.D.) of leaves and branches of different ages.

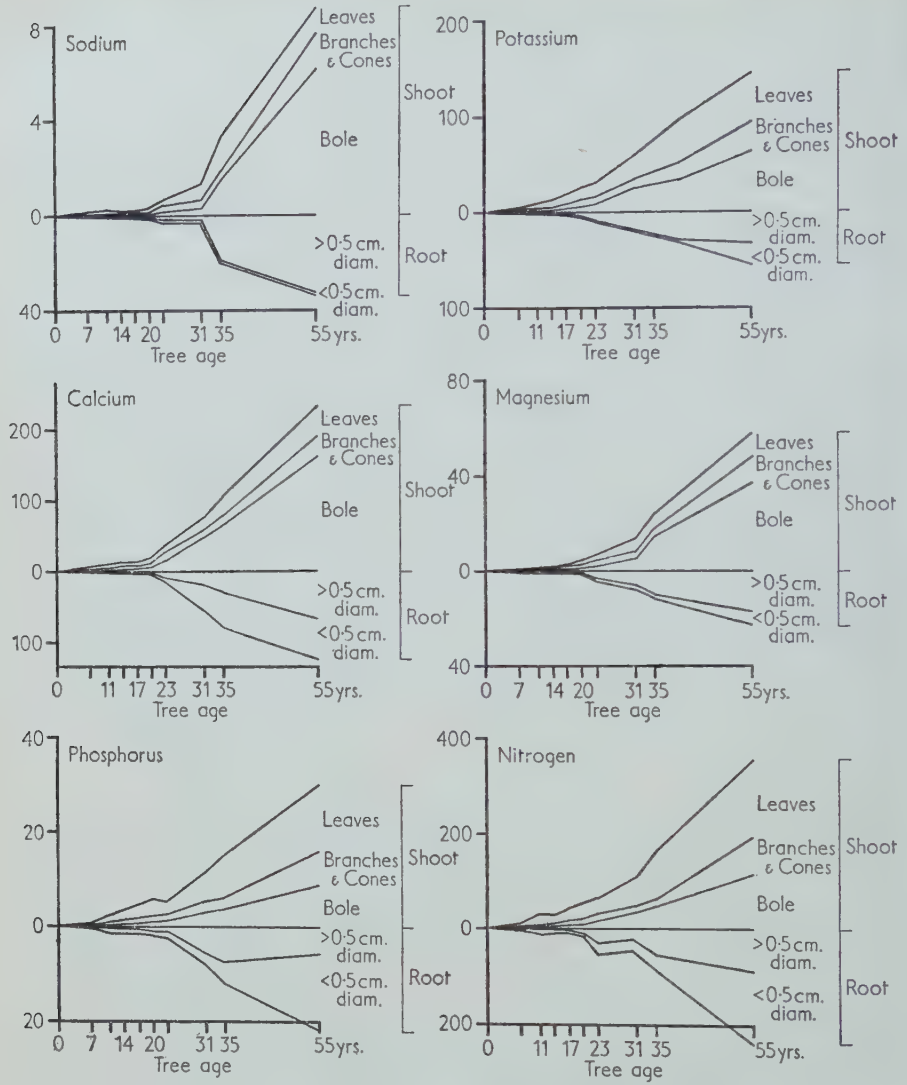


FIG. 3. The nutrient content (g.) of average trees. N.B. The scale for sodium in the shoots is less than for the roots.

to the decreasing stocking density in the older plantations the total nutrient content of all the living trees in the stands (Fig. 4) does not continue to increase so rapidly as that for the individual trees. In fact, the nutrient content of the canopy tends to be more or less constant from about twenty years onwards although the weights of nutrients in the boles and root systems increase slightly (Table 1).

By the time the plantations are 55 years of age, the amount of nutrients in the trees already harvested is greater than that in the remaining, living trees.

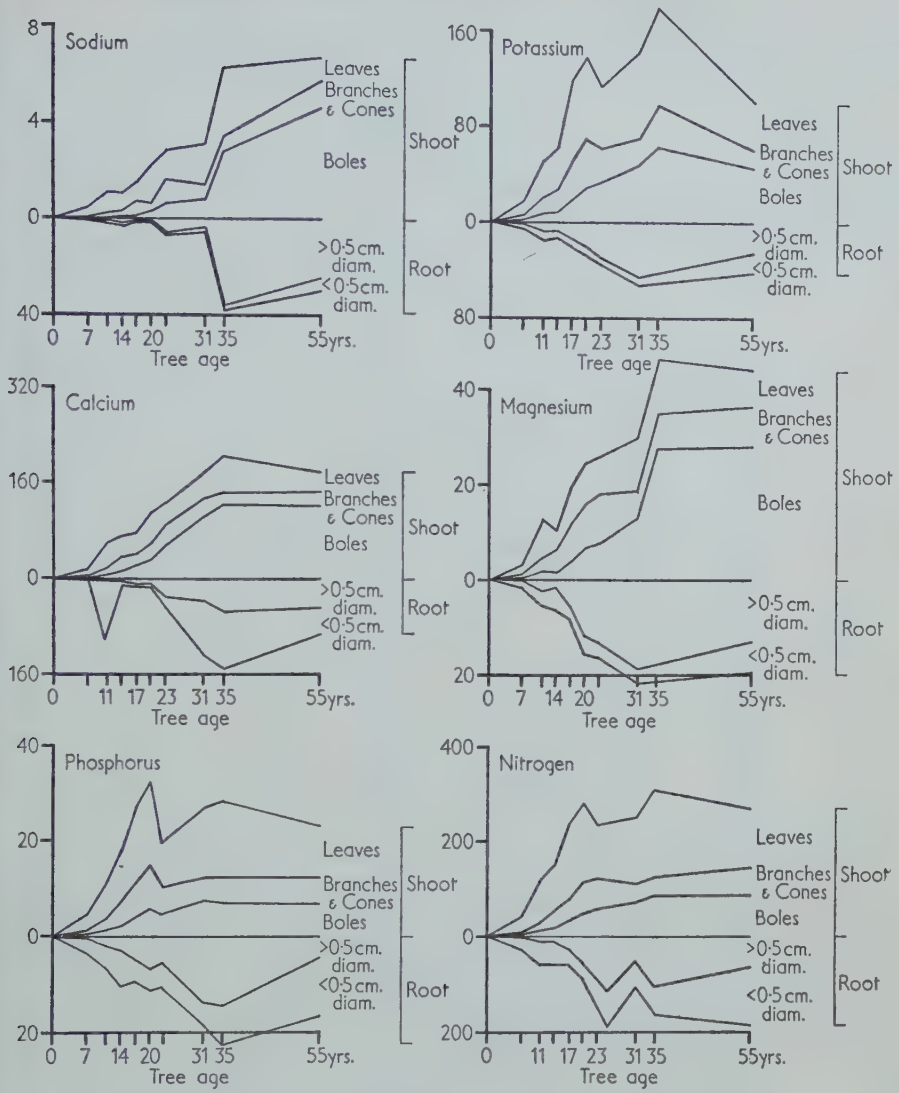


FIG. 4. The weight of nutrients (kg./ha.) contained in stands of *Pinus sylvestris*. N.B. The scale for sodium in the shoots in less than for the roots.

However, since only the tree boles are harvested, the total removal of nutrients from the site in the thinnings is relatively small, amounting to 5 kg. of sodium, 98 kg. of potassium, 210 kg. of calcium, 42 kg. of magnesium, 14 kg. of phosphorus, and 161 kg. of nitrogen per hectare after 55 years. The amounts of nutrients left on the plots in the canopies and roots of the thinnings greatly exceed that removed in the boles. If the plantations were clear felled at 55 years of age and only the boles removed, as in normal forestry management,

TABLE 1

The Nutrient Content (kg./ha.) of the Stock and Thinnings of Pinus sylvestris

Element	Age (yrs.)	Living trees				Past thinnings			
		Canopy	Bole	Roots	Total	Canopy	Bole	Roots	Total
Na	7	0·5	0·1	0·4	0·9	—	—	—	—
	11	1·0	0·1	1·9	3·0	—	—	—	—
	14	0·9	0·1	3·0	4·0	—	—	—	—
	17	1·4	0·2	0·9	2·5	—	—	—	—
	20	1·9	0·3	0·9	3·1	—	—	—	—
	23	2·3	0·6	6·6	9·5	1·0	0·2	1·7	2·9
	31	2·3	0·8	5·2	8·3	2·1	0·5	4·5	7·1
	35	3·5	2·8	37·1	43·4	2·9	0·9	9·8	13·6
	55	2·1	4·6	29·7	36·4	6·3	5·2	41·4	52·9
K	7	16	3	7	26	—	—	—	—
	11	43	8	15	66	—	—	—	—
	14	52	10	13	75	—	—	—	—
	17	95	19	21	135	—	—	—	—
	20	110	29	27	166	—	—	—	—
	23	81	34	35	150	39	13	13	65
	31	94	49	53	196	80	32	35	147
	35	116	64	61	241	104	45	48	197
	55	61	46	43	150	187	98	92	377
Ca	7	12	2	4	18	—	—	—	—
	11	51	7	105	163	—	—	—	—
	14	60	12	10	82	—	—	—	—
	17	57	19	14	90	—	—	—	—
	20	79	31	16	126	—	—	—	—
	23	71	56	48	175	37	19	14	70
	31	76	104	131	311	78	56	71	205
	35	80	124	151	355	100	83	108	291
	55	57	122	93	272	175	210	211	596
Mg	7	3	< 1	2	5	—	—	—	—
	11	11	2	5	18	—	—	—	—
	14	9	2	5	16	—	—	—	—
	17	16	4	6	26	—	—	—	—
	20	18	7	8	33	—	—	—	—
	23	18	8	16	42	8	3	5	16
	31	17	13	17	47	17	8	13	38
	35	19	28	23	70	22	13	18	53
	55	16	28	20	64	43	42	37	122
P	7	4	1	4	9	—	—	—	—
	11	10	1	7	18	—	—	—	—
	14	16	2	11	29	—	—	—	—
	17	23	4	10	37	—	—	—	—
	20	27	6	11	44	—	—	—	—
	23	15	5	11	31	9	2	5	16
	31	20	8	18	46	18	5	12	35
	35	21	7	23	51	23	7	17	47
	55	17	7	17	41	42	14	33	89
N	7	38	4	27	69	—	—	—	—
	11	110	11	61	182	—	—	—	—
	14	135	17	61	213	—	—	—	—
	17	206	33	62	301	—	—	—	—
	20	233	48	88	369	—	—	—	—
	23	177	59	189	425	81	22	60	163
	31	180	73	109	362	128	52	122	302
	35	221	86	164	471	162	70	154	386
	55	181	88	184	453	364	161	340	865

the total removal of nutrients as kg./ha. for the whole period would be 9·8 of sodium, 144 of potassium, 332 of calcium, 70 of magnesium, 21 of phosphorus, and 249 of nitrogen, giving mean annual averages for the different elements of 0·2, 2·6, 6·0, 1·3, 0·4, and 4·5 respectively.

Dead branches may persist on the living trees for several years, depending on factors such as exposure to wind and intensity of pruning. Fairly large amounts of plant nutrients are contained in the dead branches (Table 2), but the amounts are rather variable and have not been included in Table 1 which refers solely to the living parts of the trees.

TABLE 2

The Nutrient Content (kg./ha.) of Dead Branches retained on Living Trees

Age (yrs.)	Na	K	Ca	Mg	P	N
7	0	0	0	0	0	0
11	< 0·1	< 0·1	0·2	< 0·1	< 0·1	0·2
14	< 0·1	0·5	4·0	0·9	0·3	5·8
17	0·1	1·1	9·4	0·8	0·8	10·3
20	0·2	3·2	18·2	1·9	2·0	28·4
23	0·4	4·4	15·5	2·2	2·5	44·0
31	0·3	2·6	23·1	4·2	1·3	22·0
35	0·6	2·4	9·0	2·0	1·5	29·7
55	0·8	2·1	8·9	3·5	0·8	16·1

4. *Total nutrient uptake*

The nutrient content of the standing stock does not represent the total nutrient uptake since, apart from losses due to the progressive thinning and cropping, some parts of the trees, e.g. leaves and branches, are shed regularly as the trees grow. Because the trees form an age series it has been possible to make an estimate of the gross uptake of nutrients by the trees at different ages (Table 3). The values obtained are underestimates since the loss of nutrients by bark shedding, root decay, and through leaching by rain water (Madgwick and Ovington, in press) are not included. The mean and current annual uptake of nutrients by the trees are given in Fig. 5.

Apart from sodium, the current annual uptake of nutrients tends to reach a maximum at about 20–23 years of age, i.e. just at the time of the first thinning when the stands are dense and the crowns form a fairly complete cover over the ground. The maximum current annual uptake of nutrients, as kg./ha., amounts to 11 for sodium, 65 for potassium, 83 for calcium, 18 for magnesium, 13 for phosphorus, and 180 for nitrogen.

The change in mean annual uptake of nutrients with age is much more regular for the current values and shows an initial period in which nutrient uptake increases steadily up to about 30 years of age, after which it remains relatively constant at approximately 2 kg./ha. of sodium, 33 of potassium, 41 of calcium, 7 of magnesium, 7 of phosphorus, and 78 of nitrogen.

TABLE 3

Accumulated Nutrient Uptake (kg./ha.) by Tree Stands including Thinnings

Element	Age (yrs.)	Leaves	Cones	Branches	Boles	Roots	Total
Na	7	1·1	0	0·1	0·1	0·4	1·7
	11	3·1	0	0·3	0·1	1·9	5·4
	14	5·0	< 0·1	0·4	0·1	3·0	8·5
	17	6·6	< 0·1	0·9	0·2	0·9	8·6
	20	8·1	< 0·1	1·2	0·3	0·9	10·5
	23	11·0	0·1	2·6	0·8	8·3	22·8
	31	20·2	0·1	4·0	1·3	9·7	35·3
	35	24·0	0·1	5·2	3·7	46·9	79·9
	55	44·3	0·4	6·0	9·8	71·1	131·6
K	7	13	0	4	3	7	27
	11	45	0	19	8	15	87
	14	95	< 1	30	10	13	148
	17	166	5	60	19	21	271
	20	233	22	80	29	27	391
	23	308	30	153	47	48	586
	31	549	36	196	81	88	950
	35	676	45	242	109	109	1,181
	55	1,286	102	266	144	135	1,933
Ca	7	19	0	2	2	4	27
	11	75	0	13	7	105	200
	14	168	< 1	25	12	10	215
	17	249	< 1	55	19	14	337
	20	312	1	70	31	16	430
	23	399	2	140	75	62	678
	31	650	2	198	160	202	1,212
	35	758	3	242	207	259	1,469
	55	1,367	7	262	332	304	2,272
Mg	7	2	0	1	< 1	2	5
	11	11	0	4	2	5	22
	14	24	< 1	6	2	5	37
	17	35	< 1	14	4	6	59
	20	44	2	19	7	8	80
	23	58	3	41	11	21	134
	31	103	3	54	21	30	211
	35	121	4	65	41	41	272
	55	222	10	72	70	57	431
P	7	3	0	1	1	4	9
	11	11	0	4	1	7	23
	14	26	< 1	7	2	11	46
	17	45	1	14	4	10	74
	20	61	4	19	6	11	101
	23	77	6	34	7	16	140
	31	122	7	43	13	30	215
	35	146	9	51	14	40	260
	55	265	21	56	21	50	413
N	7	33	0	5	4	27	67
	11	135	0	26	11	61	233
	14	325	1	44	17	61	448
	17	533	8	95	33	62	731
	20	715	30	126	48	88	1,007
	23	912	44	260	81	249	1,546
	31	1,458	53	344	125	231	2,211
	35	1,734	62	408	156	318	2,678
	55	3,467	126	451	249	524	4,817

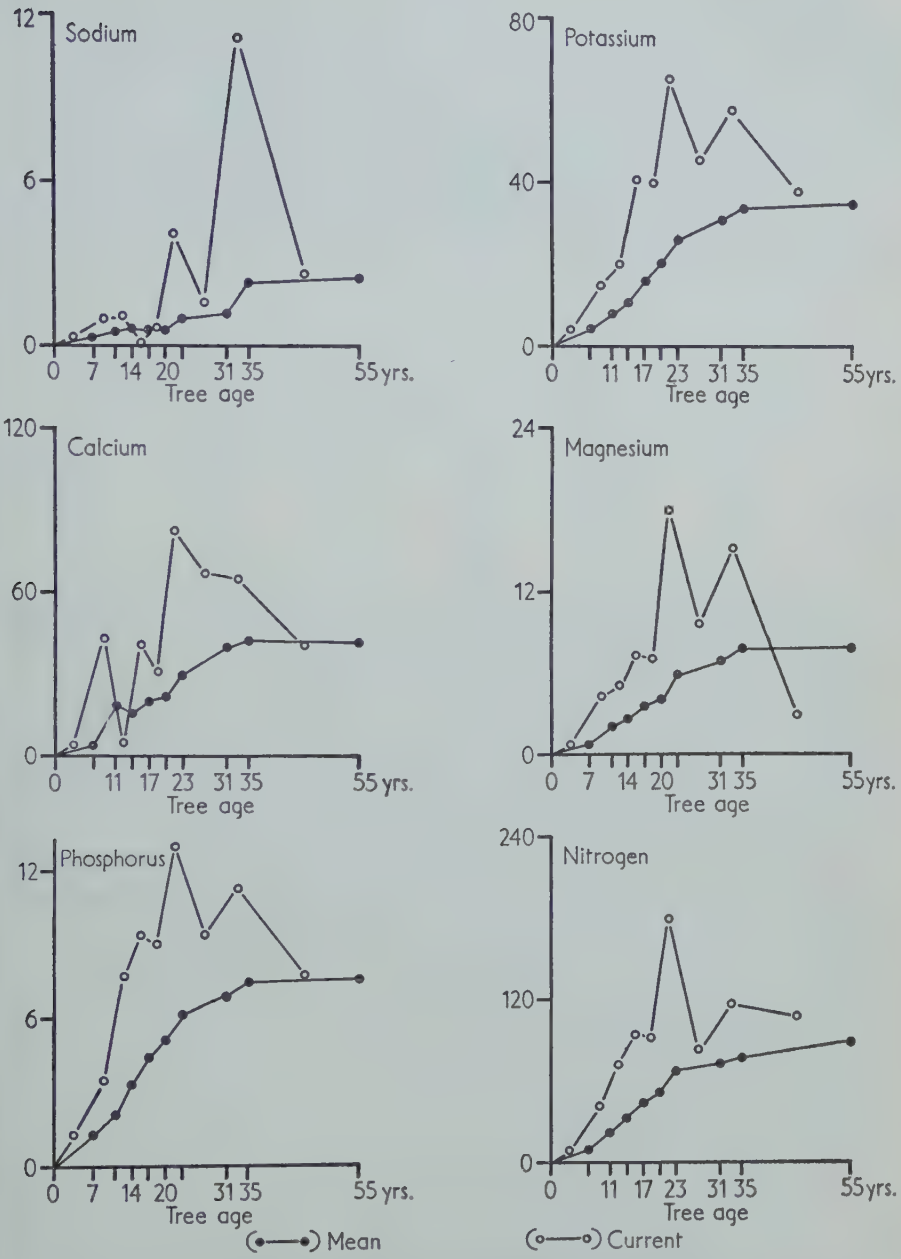


FIG. 5. The mean and current annual uptake (kg./ha.) by the trees in plantations of *Pinus sylvestris*.

Discussion

The changes recorded in the percentage composition of the trees are in general agreement with data obtained by others and can be related to the gradual maturation of the trees. The interpretation of changes in percentage composition in terms of the mass circulation of nutrients is difficult. For example, the fascicles, besides changing their chemical composition during the three-year life period, also increase in weight. The increase in weight of each fascicle with age offsets to some extent the decreasing percentages of potassium, phosphorus, and nitrogen so that the amounts of these three elements per fascicle tends to be relatively constant from the end of the first

Table 4

Annual Nutrient Uptake (kg./ha.) by Seven Common Agricultural Crops (from Russell, 1950)

	Sodium	Potassium	Calcium	Magnesium	Phosphorus	Nitrogen
Max.	99	280	72	29	26	167
Min.	2	27	3	4	6	52
Av.	18	93	29	11	13	93

year. In contrast, the increase in the total content of sodium and calcium per fascicle is much greater than would be anticipated from the increase in the percentage values, because of the dry-weight increase.

It seems surprising that the differences in soil properties, e.g. of soil calcium, between plots have had so little effect on the chemical composition of the trees and on the build-up of nutrients within the trees. Comparisons of the results given in this paper with those of other workers also seems to show little relationship between the abundance of nutrients in the soil and the amounts incorporated in the trees, but more extensive surveys over a wide range of soil types are needed to test this. Wright and Will (1958) have estimated the nutrient content of Scots pine at three different ages growing on acid dune sand with a low calcium content (Ovington, 1950). The nutrient content of the pine trees growing on the acid sand is almost identical to that for trees of comparable age growing on the more calcareous sandy soils of Thetford Chase. *Pinus sylvestris* is usually not regarded as a demanding tree species so that it seems likely that its nutrient requirements can be satisfied on soils of low fertility and that there is little tendency for excessive, luxury accumulation of nutrients.

Unfortunately, it has not been possible to obtain data of the uptake of nutrients by agricultural crops growing on soils similar to those on which the trees are growing, but broad comparisons can be made between the nutrient uptake of the trees and that of seven typical agricultural crops in England (Table 4). Considering the mean annual absorption of elements by the pine trees over the whole 55-year period, nutrient uptake is relatively small and only the calcium value is greater than the average for the agricultural crops.

The estimates of the mean annual uptake of nutrients by the trees include the juvenile stages when true woodland conditions were not established. When tree growth is at a maximum, the current annual uptakes of calcium, magnesium, and nitrogen by the trees is greatly in excess of the average values for the agricultural crops whilst the values for phosphorus are about the same. Compared with other herbaceous vegetation types, the annual uptake of plant nutrients by an actively growing Scots pine woodland may be relatively large and it seems likely that other more demanding tree species will have even higher nutrient absorption rates than *Pinus sylvestris*.

TABLE 5

The Return of Nutrients (kg./ha.) to the Soil from the Trees (inclusive of Dead Branches on Trees)

After	Na	K	Ca	Mg	P	N
7 yrs.	0·8	1	9	> 1	> 1	> 1
11 yrs.	2·4	21	37	4	5	51
14 yrs.	4·5	73	133	21	17	235
17 yrs.	6·1	136	247	33	37	430
20 yrs.	7·4	225	304	47	57	638
23 yrs.	13·1	423	484	89	107	1,099
31 yrs.	26·5	722	845	156	164	1,797
35 yrs.	35·6	895	1,031	189	202	2,137
55 yrs.	90·0	1,685	1,790	325	358	4,203

Normally, woodlands are not manured regularly so that nutrient absorption by the trees would seem to represent a serious drain on the soil resources. However, only a small proportion of the nutrients absorbed by the trees are retained within the crop (Table 5), and only a small part of this is removed when the tree boles are harvested. At 55 years of age, the total nutrient content of the standing trees and the boles of the trees that have been cropped represent only 32 per cent. of the sodium, 13 per cent. of the potassium, 21 per cent. of the calcium, 25 per cent. of the magnesium, 13 per cent. of the phosphorus, and 13 per cent. of the nitrogen taken up by the trees during their lifetime. The rest of the nutrient uptake has been returned to the soil either as litter fall or as canopy and root material from the harvested trees. Nevertheless, the increasing amounts of plant nutrients incorporated within the trees as they become older does represent a significant removal of the elements from the active nutrient cycle between the soil and trees.

Not all of the nutrients returned to the soil are available immediately for reabsorption since an organic layer accumulates over the surface of the mineral soil and the large roots decompose slowly. The rate of decay and build-up of the different components of the trees within the surface humus varies considerably, and changes in the ground vegetation also affect the overall circulation of nutrients within the woodlands.

Summary

1. The percentages of sodium, potassium, calcium, magnesium, phosphorus, and nitrogen in the leaves, branches, cones, boles, and roots of sample trees from an age series of nine plantations of Scots pine have been determined.

2. There is considerable variation within each component of the tree in the proportions of the different elements.

3. The total weights of each element per tree and in all the trees of each plantation have been determined.

4. The gross uptake of nutrients, the retention of nutrients within the living trees, the removal of nutrients from the site in harvested tree stems, and the return of nutrients to the soil from the trees have been calculated.

5. Comparisons are made between the nutrient uptake of tree stands and typical agricultural crops.

Literature Cited

Madgwick, H. A. I., and Ovington, J. D., 1959: The Chemical Composition of Precipitation in Adjacent Forest and Open Plots. Forestry. (In press.)

Ovington, J. D., 1950: The Afforestation of the Culbin Sands. J. Ecol. **38,** 303.

—— 1957*a*: The Volatile Matter, Organic Carbon and Nitrogen Contents of Tree Species grown in Close Stands. New Phytol., **56,** 1.

—— 1957*b*: Dry-matter Production by *Pinus sylvestris* L. Ann. Bot., N.S., **21,** 287.

—— and Madgwick, H. A. I., 1959: The Sodium, Potassium and Phosphorus Contents of Tree Species grown in Close Stands. New Phytol. (In press.)

—— The Calcium and Magnesium Contents of Tree Species grown in Close Stands. New Phytol. (In press.)

Piper, C. S., 1950: Soil and Plant Analysis. Monograph from the Waite Agricultural Research Institute, Adelaide, Australia.

Russell, E. J., 1950: Soil Conditions and Plant Growth. London.

Wright, T. W., and Will, G. M., 1958: The Nutrient Content of Scots and Corsican Pines growing on Sand Dunes. Forestry, **31,** 13.

The Carbohydrate Nutrition of Tomato Roots

VII. Sugars, Sugar Phosphates, and Sugar Alcohols as Respiratory Substrates for Excised Roots

BY

D. R. MORGAN AND H. E. STREET

(*Department of Botany, University College of Swansea*)

With three Figures in the Text

ABSTRACT

The respiratory responses of substrate-depleted excised root tips to a range of sugars, sugar phosphates, and sugar alcohols have been determined by measuring oxygen uptake by the direct method of Warburg.

Sucrose, dextrose, and laevulose are the only sugars which promote a high level of oxygen uptake.

The effects of azide and DNP on the oxygen uptake promoted by sucrose and by dextrose are described.

Mannose is a strong inhibitor of respiration. This inhibition is reversed by the simultaneous addition of those sugars which also reverse the growth inhibition caused by mannose. Mannose inhibits the respiration of sucrose and of glycolytic intermediates. Galactose is slowly respired and does not, even at high concentration, inhibit the respiration of sucrose.

The results are discussed in relation to the growth effects of the sugars tested.

INTRODUCTION

KNOWLEDGE of the carbohydrate metabolism of excised tomato roots is essential if we are to explain the unique activity of sucrose as a source of carbon and energy for root growth (Dormer and Street, 1949; Ferguson, Street, and David, 1958*a*), the inhibitory activities of galactose and mannose, and the reversal of their growth inhibitions by the simultaneous presence of dextrose or xylose (Ferguson, Street, and David, 1958*b*). With this in mind a study has now been made of the ability of sugars, sugar phosphates, and sugar alcohols to act as respiratory substrates when supplied to carbohydrate-depleted excised roots.

EXPERIMENTAL

General Experimental Procedures

A clone of excised tomato roots derived from a single seed of the doubled-haploid variety San Marzano was used throughout. The general cultural technique was as described in earlier papers (Street and Lowe, 1950; Street and McGregor, 1952). The standard culture medium contained 1·5 per cent. sucrose, and ferric chloride as the iron source (White, 1943; Ferguson, Street, and David, 1958*a*).

Oxygen uptake was determined by the direct method of Warburg at a bath temperature of 27° C. Each Warburg flask was charged with 2 ml. of standard

[Annals of Botany, N.S. Vol. 23, No. 89, 1959.]

medium minus sucrose (minus-sugar medium). The substrate was contained in the side arm as 0·5 ml. aqueous solution (pH adjusted, when necessary, by NaOH to 4·8–5·0, e.g. in the case of the sugar phosphates). Except where otherwise stated the root tips were allowed to respire in the Warburg vessels for 9 hrs. (−9 to 0 hrs.) prior to the addition of substrate, and for 10 hrs. (0 to + 10 hrs.) following this addition. Readings were taken at 2-hr. intervals during the test period. At the conclusion of the experiment the root tips

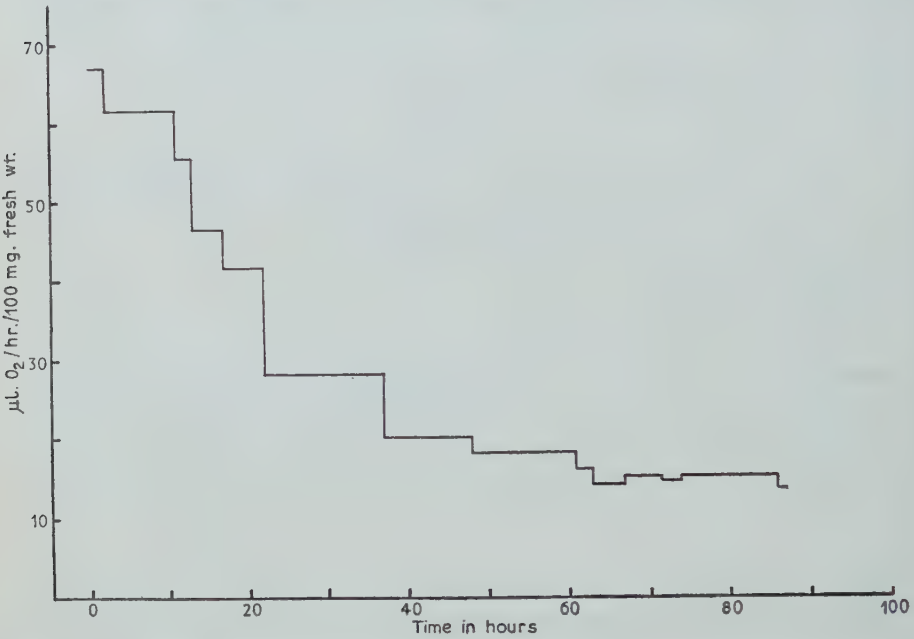


FIG. 1. The change in the rate of oxygen uptake of 20-mm. root tips suspended from the time of excision (0 hrs.) in minus-sugar medium.

were removed, blotted, and their fresh weight determined. Oxygen uptake is expressed as µl./hr./100 mg. final fresh weight. Each treatment was represented by two replicate flasks. Values for the Respiratory Quotient (R.Q.) were determined by the use of replicate vessels with no CO_2 absorbent.

The standard method for the preparation of root material was as follows: 7-day 'sector' cultures (each with 5 or 6 main laterals) were washed with sterile minus-sugar medium and transferred, with aseptic precautions, to flasks containing 25 ml. of this minus-sugar medium and incubated at 27° for 51 hrs. (−60 to −9 hrs.). Main lateral tips (20 mm.) were then excised and 30 such tips placed in the suspending medium in each Warburg flask.

The fall in respiration rate during starvation

20-mm. tips, excised from 7-day 'sector' cultures and washed in sterile minus-sugar medium, were transferred directly to Warburg flasks and their O_2 uptake followed for 86 hrs. (Fig. 1). Between 40 and 86 hrs. the rate

remained steady at between 18 and 15 μl/hr. The standard period of 60-hr. starvation (51 hrs. before and 9 hrs. after excision of tips) was chosen on the basis of these preliminary studies.

The ability of various sugars, sugar phosphates, and sugar alcohols to act as respiratory substrates

The results of a general survey of the ability of sugars, sugar phosphates, and sugar alcohols to act as respiratory substrates are summarized in Table 1. Sucrose, dextrose, and laevulose were the only substances which promoted a high rate of oxygen uptake. Galactose, maltose, glucose-1-phosphate, and glucose-6-phosphate promoted a lower rate of oxygen uptake. The enhancement of respiration by the glucose phosphates could not be reproduced by the application of the potassium or orthophosphate concentrations introduced by the acid esters. Mannose reduced the oxygen uptake below that of the 'endogenous' respiration (respiration of the control root tips in minus-sugar medium). The remaining compounds tested were inactive, with the possible exception of raffinose and xylose which, at a concentration of 2 per cent., just prevented the fall in rate of oxygen uptake which occurs, during the experimental period, in the absence of substrate.

In order to test the effect of a longer exposure to selected substrates, the 7-day 'sector' cultures and the 20 mm. tips after excision were submitted to the test substrate during the 60-hr. period which normally precedes addition of substrate from the side arm. The results obtained (Table 2) confirmed the high rate of respiration in sucrose and dextrose and showed that the lower rate of oxygen uptake with galactose was maintained. Raffinose which did not give clear-cut stimulation in the short-period test now showed a rate of oxygen uptake above the control. The inability of mannitol to stimulate respiration was confirmed. *L*-arabinose, which during shorter exposures is without effect on oxygen uptake, did now at the highest concentration tested (2·0 per cent.) partially inhibit the endogenous respiration. A similar inhibition of the endogenous respiration was recorded with mannose down to the lowest concentration tested (0·02 per cent.).

The respiration of sucrose, dextrose, and laevulose

The uniformly high rate of oxygen uptake achieved in the presence of sucrose or dextrose or laevulose is in contrast to the marked superiority of sucrose over either monosaccharide as a carbon source for root growth (Dormer and Street, 1949; Ferguson, Street, and David, 1958*a*).

Using tips obtained by the normal 'starvation' technique, Respiratory Quotient (R.Q.) values were determined for the endogenous respiration and for respiration following the addition of selected substrates (Table 3). The low R.Q. values during the later stages of starvation suggest marked depletion of carbohydrates and the very low value in presence of mannose suggests a complete inhibition of carbohydrate respiration. The high values with sucrose and dextrose point to a complete oxidative respiration of both sugars.

TABLE I

The Ability of Various Compounds to Promote the Oxygen Uptake of Starved Root Tips. Test Substance added at 0 hrs.

Substrate	Concentration (per cent.)	O_2 uptake μl./hr./100 mg. fresh wt.	
		−9 to 0 hrs.	+8 to +10 hrs.
Sucrose	0·0*	24	16
	0·1	20·5	27·5
	0·4	20·5	45
	2·0	19·5	59
Dextrose	0·0	18	13
	0·1	17·5	32
	0·4	18·5	42
	2·0	19·5	48
Laevulose	0·0	21·5	13·5
	0·1	18·5	24·5
	0·4	17·5	40·5
	2·0	20	55
d-galactose	0·0	19·5	13
	0·1	18·5	25·5
	0·4	20·0	29
Maltose	0·0	16·5	13·5
	0·4	17·0	18·5
	2·0	16·5	27·5
Raffinose	0·0	16·0	11·0
	2·0	15·5	20
d-xylose	0·0	16·5	6
	0·4	16·5	14·5
	2·0	17	21·5
d-ribose	2·0	18·5	14·5
l-arabinose	0·4	12	13
Sorbose	2·0	20	17·5
Rhamnose	2·0	14·5	13
Melibiose	0·4	18	13·5
Lactose	0·4	14·5	10·5
d-mannose	0·0	20	10
	0·4	19·5	7·5
	2·0	20	5·5
Dulcitol	0·4	12·0	9·0
Mannitol	0·4	18·5	13·5
Sorbitol	0·4	17·5	17·7
Glucose-1-phosphate	0·0	16	13·5
	0·4†	17·5	33
Glucose-6-phosphate	0·0	17·5	15·5
	0·4†	17	37·5
Fructose-1-6-diphosphate	0·4†	19·5	22·5
Mannose-6-phosphate	0·0	19	13
	0·4†	18	12·5
d-mannose	0·4	18	7

* No substrate control = 'endogenous' rate of O_2 uptake.

† Concentration to give the equivalent carbon to 0·4 per cent. of hexose.

TABLE 2

Oxygen Uptake after 68 hrs. Exposure to the Test Substrate

Substrate	Concentration per cent.	O_2 uptake μl./hr./100 mg. fresh wt. 66–68 hrs.
Sucrose	0·0	18
	0·02	33
	0·1	56
	0·4	70·5
	2·0	84·5
Dextrose	0·0	13·5
	0·02	31
	0·1	55
	0·4	60·5
	2·0	67
d-galactose	0·0	18
	0·4	25·5
	2·0	31·5
Raffinose	0·0	13
	0·4	23·5
	2·0	33
Mannitol	0·0	11
	0·4	12·5
	2·0	11·5
l-arabinose	0·0	16
	0·4	16·5
	2·0	10
d-mannose	0·0	20·5
	0·02	13
	0·4	12

TABLE 3

Values for the Respiratory Quotient (R.Q.) with various Substrates (2 per cent. Concentration)

Substrate	R.Q.	Period during which determined
Endogenous respiration (no substrate addition)	0·80–0·83	−9 to 0 hrs.
	0·70–0·76	+5 to 10 hrs.
Sucrose	0·96	,,
Dextrose. . . .	0·92	,,
d-galactose . . .	0·90	,,
Raffinose . . .	0·91	,,
Mannitol . . .	0·64	,,
d-mannose . . .	0·60	,,

When the 7-day 'sector' cultures and the 20-mm. tips following excision were submitted during the 60-hr. pretreatment period to media containing 2 per cent. sucrose, dextrose or laevulose, they had a high rate of oxygen uptake at 0 hrs. The oxygen uptake of such tips has also been followed during a further 10 hrs. after transfer to a minus-sugar medium (Table 4). Clearly the rate of oxygen uptake falls more rapidly in tips which have been pretreated

with the monosaccharides than in those pretreated with sucrose. This suggests that despite the high oxygen uptake in the presence of an external supply of the monosaccharides there is a failure to build up from them an endogenous reserve of actively respirable material. This suggested that the respiration of these monosaccharides and of sucrose might not occur at the same site in the root cells or that the monosaccharide respiration might be less effectively linked to oxidative phosphorylation than is sucrose respiration.

TABLE 4

Oxygen Uptake During Starvation of Root Tips Supplied During the Previous 60 hrs. with Substrate (2 per cent. Concentration)

Substrate	O_2 uptake μl./hr./100 mg. fresh wt. during starvation period	
	0–2 hrs.	6–8 hrs.
Sucrose . . .	80	82
Dextrose . . .	79	52·5
Laevulose . . .	84	58

TABLE 5

Oxygen Uptake of Roots Supplied with Sucrose, Dextrose, or Laevulose in Media at pH 4·8 and at pH 6·6

Substrate	Initial pH of external medium	O_2 uptake μl./hr./100 mg. fresh wt.	
		−9 to 0 hrs.	+8 to +10 hrs.
Sucrose	4·8	18	43
	6·6	19·5	44·5
Dextrose	4·8	19·5	37·5
	6·6	18·5	41·5
Laevulose	4·8	19·5	38
	6·6	18·5	37·5

In view of the evidence (Rothstein, 1954) for the surface localization in certain cells of enzymes concerned in respiration and absorption, the sensitivity of sucrose, dextrose, and laevulose respirations to external pH was first tested using media of initial pH 4·8–5·1 and pH 6·6 (calcium carbonate used as buffer). Oxygen uptake was for each substrate the same at both pH values (Table 5).

Stenlid (1948) has shown that inhibition of the endogenous respiration of wheat roots by sodium azide is very markedly reduced when the pH of the external solution approaches neutrality. Since hydrazoic acid is a weak acid with pK = 4·6–4·8 and is therefore 99 per cent. dissociated at pH 6·7 Stenlid has suggested that only the undissociated acid may be able to penetrate root cells with any rapidity. From his observation that at pH 7, 10^{-3} M. NaN_3 did not inhibit the endogenous respiration of wheat roots but did prevent the rise in oxygen uptake which would otherwise have followed addition of 0·01 M. dextrose, Stenlid concluded that azide either inhibited a surface-located respiratory system or a phosphorylating process involved in dextrose absorption.

Using a medium buffered at pH 6·9–7·2 by addition of calcium carbonate, a study has been made of the effects of sodium azide over the concentration range 10^{-3} to 10^{-1} M., on the 'endogenous', sucrose, and dextrose respirations of tomato root tips. 10^{-3} M. NaN_3 had little effect on the 'endogenous' respiration and did not prevent the normal rise in respiration when either sucrose or dextrose were added simultaneously; $10^{-1.5}$ M. NaN_3 which by the end of the 10-hr. experimental period slightly inhibited 'endogenous' respiration, completely prevented the rise in respiration normally accompanying addition of either of the sugars (Table 6). The respirations of sucrose and dextrose therefore did not differ in their sensitivity to azide.

TABLE 6

The Effect of NaN_3 on the Respiration of Roots in the Absence and in the Presence of Sucrose and Dextrose (Calcium Carbonate Buffer)

Treatment at 0 hrs.	O_2 uptake µl./hr./100 mg. fresh wt.	
	−9 to 0 hrs.	+8 to +10 hrs.
No addition	18	13
10^{-3} M. NaN_3	15·5	13
2% sucrose	16	35
2% sucrose + 10^{-3} M. NaN_3	16	32
2% dextrose	16·5	30·5
2% dextrose + 10^{-3} M. NaN_3	18	31·5
No addition	20	19
$10^{-1.5}$ M. NaN_3	24	11
2% sucrose	20	49
2% sucrose + $10^{-1.5}$ M. NaN_3	22·5	24
2% dextrose	19	39
2% dextrose + $10^{-1.5}$ M. NaN_3	20	15·5

The stimulation of oxygen uptake by low concentrations of 2·4-dinitrophenol (DNP) arises from an increase in the rate of glycolysis (Beevers, 1953). Work, mainly with cell-free systems, has shown that DNP uncouples oxidation from phosphorylation. Growth and other energy-requiring processes are more sensitive to inhibition by DNP than is oxygen uptake; growth is often inhibited by concentrations which stimulate oxygen uptake (Simon, 1953). If the effectiveness of sucrose as a carbon source for excised tomato root growth arises from the effective coupling of its oxidation to phosphorylation, then it would be expected that its degradation would be limited by the level of P-acceptors (e.g. ADP) and that DNP by promoting ATP breakdown would markedly enhance oxygen uptake (Hunter, 1951; Witter and Newcomb, 1952). By contrast if dextrose and laevulose respirations fail to make available an adequate supply of high-energy phosphate compounds for use in growth then the relative ineffectiveness of these monosaccharides as carbon sources would be explained. In such a circumstance it would be expected that the rate of glycolysis would not be limited by saturation of the available P-acceptors and that the maximum DNP-stimulation of oxygen uptake achieved with these monosaccharides as substrates would be less than with

sucrose. At pH 7·0–7·2 optimum stimulation of the oxygen uptake of excised tomato roots was obtained with $10^{-3.5}$ M. DNP. The influence of this concentration of DNP on the oxygen uptake of 'starved' roots and of roots supplied with sucrose, dextrose, and laevulose was therefore examined in two experiments, the results of which are shown in Table 7. In the first of these 'starved' roots were used and the DNP and carbohydrate both added at 0 hrs. In the second experiment the roots received carbohydrate during the 60-hr. pretreatment and during the experimental period. Following DNP addition the

TABLE 7

The Stimulation of Oxygen Uptake by Dinitrophenol ($10^{-3.5}$ M. DNP, pH 7·0) in the Presence of Sucrose, Dextrose, and Laevulose and in the Absence of External Carbohydrate

Pretreatment	Addition at 0 hrs.	O_2 uptake μl./hr./100 mg. fresh wt. 0 to +2 hrs.	+8 to +10 hrs.
Minus sugar	No addition	24	16
	DNP	42	18·5
	1% sucrose	30	38·5
	1% sucrose+DNP	54	41
	1% dextrose	27·5	32
	1% dextrose+DNP	56·5	43
	1% laevulose	29·5	34·5
	1% laevulose+DNP	53	38
1% sucrose	1% sucrose	65	51·5
	1% sucrose+DNP	97·5	66·5
1% dextrose	1% dextrose	66·5	54
	1% dextrose+DNP	108·8	79·5

highest rate of oxygen uptake was observed between 0 and +2 hrs., although frequently the rate at the end of the experimental period was still clearly above that recorded in the absence of DNP. Marked DNP stimulation of oxygen uptake occurred in all cases. There was no evidence of a greater stimulation in the presence of sucrose; in fact the highest stimulation was obtained with roots pretreated with dextrose.

Study of the respiratory responses to external pH, azide and DNP, therefore, failed to reveal any significant differences between the respirations of sucrose, dextrose, and laevulose.

The effect of various sugars and sugar phosphates upon the oxygen uptake associated with sucrose utilization

A study has been made of the effects of dextrose, galactose, mannose, xylose, arabinose, glucose-6-phosphate, and mannose-6-phosphate on the oxygen uptake of root tips simultaneously presented with sucrose. The sugars chosen include those most inhibitory to excised root growth (Ferguson, Street, and David, 1958*a*, *b*). The sugar phosphates were included for comparison with their corresponding monosaccharides. The results (Table 8)

show that galactose, xylose, arabinose, and the sugar phosphates do not inhibit the enhanced oxygen uptake promoted by sucrose. Mannose is, however, seen to be strongly inhibitory to sucrose respiration at a concentration of 0·02 per cent. and exerts its full inhibitory effect at 0·1 per cent.

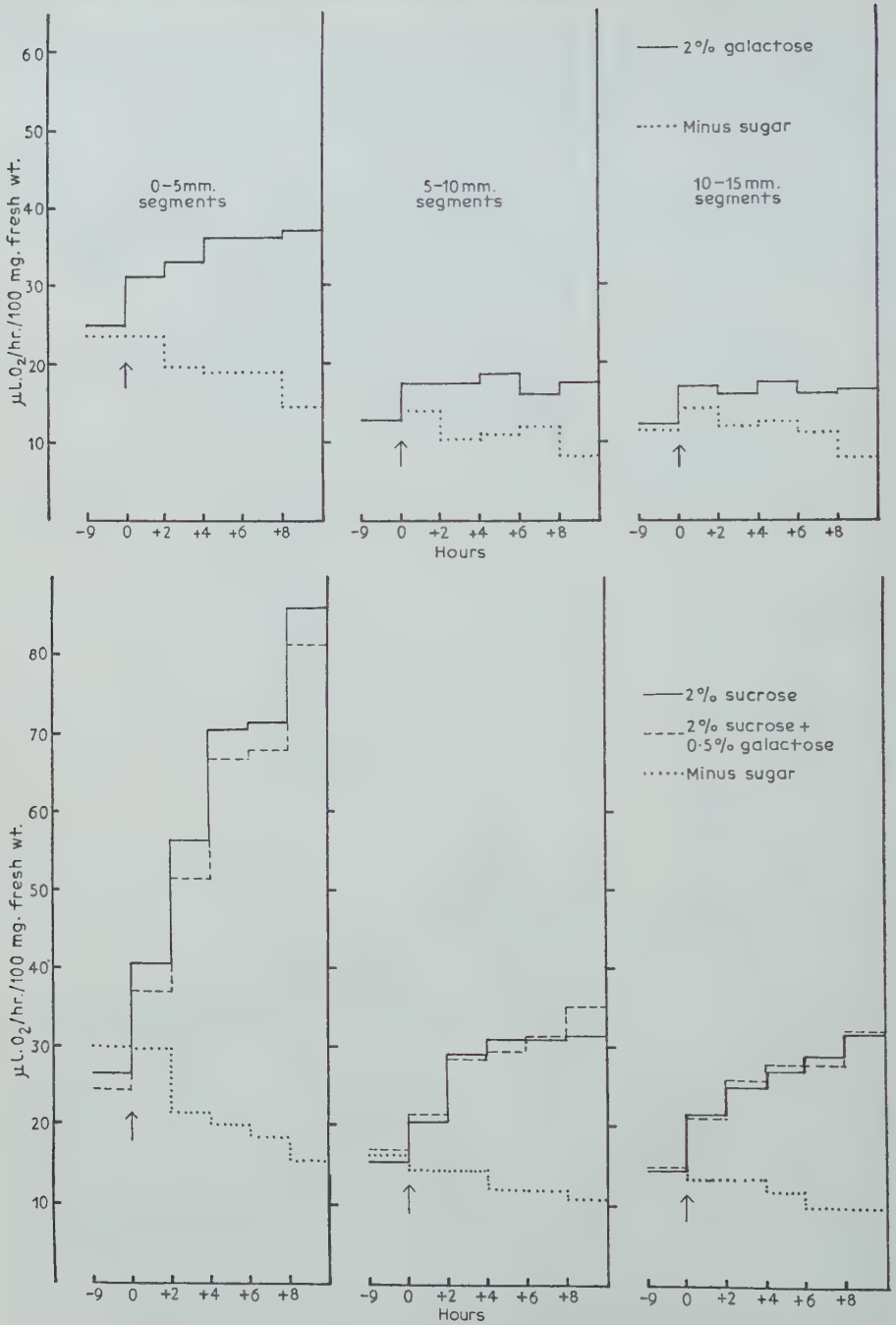
TABLE 8

The Effects of Various Sugars and Sugar Phosphates on the Oxygen Uptake of Roots Simultaneously Supplied with 2 per cent. Sucrose

Sugar or sugar phosphate	Concentration per cent.	O_2 uptake μl./hr./100 mg. fresh wt. +8 to +10 hrs.
Dextrose	0·0	45·5
	0·1	47
	0·4	47·5
	1·0	44
Glucose-6-phosphate*	0·0	48
	0·1	43·5
	0·4	37·5
d-galactose	0·0	45·5
	0·1	47·5
	0·4	46·5
d-xylose	0·0	35·5
	0·1	34
	0·4	36·5
d-arabinose	0·0	43·5
	0·1	39·5
	0·4	39
	2·0	38
d-mannose	0·0	45
	0·02	27·5
	0·1	13
	0·4	11
Mannose-6-phosphate*	0·0	49·5
	0·02	47·5
	0·1	46·5
	0·3	47

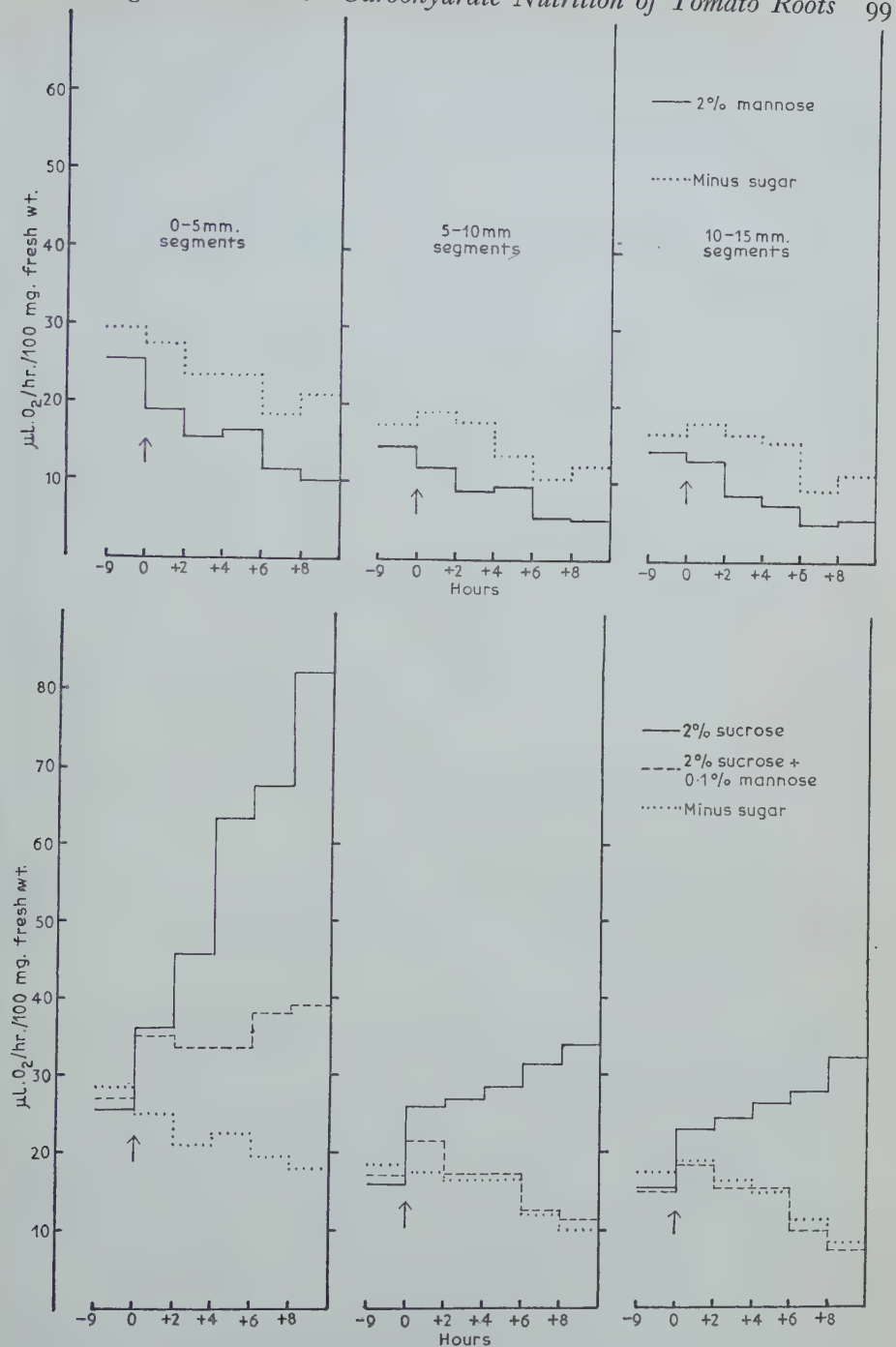
* Concentrations shown are the hexose equivalent based on carbon content.

20-mm. root tips, such as are used in the standard experiments, include not only the meristem and region of cell elongation (included within the terminal 5 mm.) but an extensive region of differentiated primary structure (Street and McGregor, 1952). Experiments were therefore undertaken to study the mannose inhibition of respiration in shorter root pieces. Segments 0–5 mm., 5–10 mm., and 10–15 mm. from the apex were cut and used, 100 pieces per Warburg vessel. Fig. 2 shows the oxygen uptake of these pieces during the 10-hr. test period when supplied with sucrose, galactose, or mannose at 2 per cent. or with the mixtures: 2 per cent. sucrose+0·1 per cent. mannose; or 2 per cent. sucrose+0·5 per cent. galactose. Mannose is inhibitory to the respiration of all three types of root segment, the oxygen



(*a*) Substrates: 2 per cent. *d*-galactose, 2 per cent. sucrose, and 2 per cent. sucrose + 0·5 per cent. *d*-galactose.

FIG. 2. The oxygen uptake of root segments excised from sector cultures. The sector cultures were grown for 7 days in standard medium and then transferred to minus-sugar medium for 51 hrs. Following excision and transfer to the Warburg vessels the root segments were sus-



(*b*) Substrates: 2 per cent. *d*-mannose, 2 per cent. sucrose, and 2 per cent. sucrose + 0·1 per cent. *d*-mannose.

pended for 9 hrs. (−9 to 0 hrs.) in minus-sugar medium, and then the test subs rate was added (as indicated by the arrow) and the oxygen uptake followed for a further 10 hrs. (0 to +10 hrs.). The 0–5-mm. segments include the root cap and apical meristem.

uptake being, in each case, lower than in the absence of any external substrate. Galactose promotes a well-marked stimulation of oxygen uptake particularly in the terminal 5-mm. pieces, and does not inhibit the high rate of respiration promoted by sucrose. By contrast mannose completely eliminates the sucrose-promoted oxygen uptake of the 5–10-mm. and 10–15-mm. segments, and markedly reduces this in the apical 5 mm. The resistance to 0·1 per cent. mannose of a fraction of the enhanced oxygen uptake due to sucrose in the terminal 5-mm. pieces may be worthy of further study in view of the

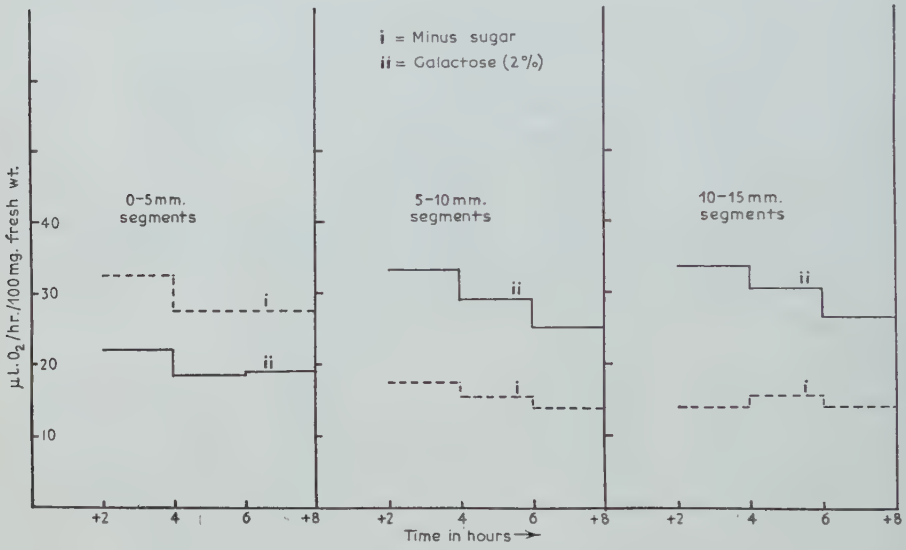


FIG. 3. The oxygen uptake of root segments:

(i) prepared as described in Fig. 2 and suspended during the test period in minus-sugar medium,

(ii) prepared as described in Fig. 2, but the sectors during the period −60 to −9 hrs. and the segments from −9 hrs. and during the test period, suspended in 2 per cent. *d*-galactose medium.

quite high survival of the capacity for subsequent growth in root apices completely inhibited by mannose (Ferguson, Street, and Davis 1958*b*).

This last observation prompted a test of the effect on respiration of pretreatment with galactose, because root tips inhibited by this sugar show a rather poor survival of their capacity for growth. 'Sector' cultures were incubated for 51 hrs. in a medium containing 2 per cent. galactose before preparation of the three kinds of 5-mm. root pieces. The oxygen uptake of such pieces in a 2 per cent. galactose medium was compared with that of similar pieces from 'starved' cultures suspended in a minus-sugar medium. The results (Fig. 3) show that the 5–10-mm. and 10–15-mm. segments receiving galactose have an enhanced oxygen uptake as compared with the minus-sugar controls but that the 0–5-mm. segments have a rate of oxygen uptake below that of 'starved' tips and that their respiration rate in galactose remains below

that of the 5–10-mm. and 10–15-mm. segments (contrast Fig. 2). Clearly the more prolonged galactose treatment has not reduced the respiratory capacity of fully expanded cells, but some impairment of meristem function is indicated by the reduced respiratory activity of the terminal 0–5-mm. pieces.

The inhibition of oxygen uptake by mannose

The inhibition of root growth by either galactose or mannose can be reversed by application of the appropriate concentration of dextrose (Ferguson, Street, and David, 1958*b*). It was, therefore, of interest to see whether the oxygen uptake with dextrose as substrate would be affected by concentrations of galactose fully inhibitory to growth, and whether the mannose inhibition of respiration would be reversed by dextrose at the minimum effective concentration required to restore growth. The oxygen uptake with 0·4 per cent. dextrose was found to be neither stimulated nor depressed by concentrations of galactose up to 2 per cent. With 2 per cent. dextrose there was no inhibition of respiration with concentrations of mannose up to 0·4 per cent.: with 0·4 per cent. dextrose there was no inhibition with mannose concentrations below 0·1 per cent., but above this concentration mannose became progressively more inhibitory with increase in its concentration. The minimum ratio of dextrose: mannose for mannose to be non-inhibitory to oxygen uptake therefore corresponded well with the minimum ratio (3·5 : 1·0) necessary to reverse completely the mannose inhibition of growth.

Tests were then made of the ability of dextrose, xylose, galactose, and laevulose to reverse the inhibition of sucrose respiration brought about by the addition of 0·1 per cent. mannose. The results are shown in Table 9. Laevulose, which will not reverse the growth inhibition due to mannose, does not antagonize the mannose inhibition of sucrose respiration. Dextrose and *d*-xylose, both of which antagonize the growth inhibition due to mannose, are able to reverse fully the depression of oxygen uptake caused by 0·1 per cent. mannose. Galactose also has activity as an antagonist of the mannose inhibition of sucrose respiration, effecting full reversal of the inhibition caused by 0·04 per cent. and partial reversal of that caused by 0·1 per cent. mannose.

These results pose the problem of the mechanism of the mannose inhibition of sucrose respiration. It could act by disrupting the phosphorylations which lead to the formation of fructose-1-6-diphosphate. The inability of mannose-6-phosphate to inhibit sucrose respiration (Table 8) suggests that mannose does not disrupt respiration by hexokinase-activated formation of this phosphate and its subsequent entry into the pathway of glycolysis. Table 10 also indicates that the respirations of laevulose, glucose-1-phosphate, and glucose-6-phosphate are strongly inhibited by concentrations of mannose which are inactive when dextrose is the substrate of respiration. None of these is therefore beyond the point of action of mannose as a respiratory inhibitor. The very low rate of oxygen uptake so far obtained with externally applied fructose-1-6-diphosphate (Table 1) precludes a critical test of the sensitivity of its respiration to mannose inhibition. We have also so far failed to obtain a

TABLE 9

The Ability of Various Sugars to Reverse the Inhibition of Sucrose Respiration caused by d-mannose

Sugar addition at 0 hrs.	O_2 uptake μl./hr./100 mg. fresh wt. −9 to 0 hrs.	+8 to +10 hrs.
No addition	19·5	14
2% sucrose	19	51·5
,, +0·1% mannose	20	20·5
,, + ,, +0·4% dextrose	20	46·5
,, + ,, +0·4% laevulose	22	25·5
No addition	19·5	16·5
2% sucrose	20	42·5
,, +0·1% mannose	18	23
,, + ,, +0·4% *d*-xylose	18·5	35·5
,, +0·4% *d*-xylose	18·5	49
,, +0·1% mannose +1·0% *d*-xylose	19·5	42·5
,, +1·0% *d*-xylose	18	47
No addition	19	16·5
2% sucrose	19	41
,, +0·04% mannose	18	31·5
,, + ,, +0·04% galactose	18	36
,, + ,, +0·1% galactose	17·5	42·5
No addition	17	14·5
2% sucrose	17·5	45
,, +0·1% mannose	15	18·5
,, + ,, +0·1% galactose	16·5	27
,, + ,, +0·4% galactose	16·5	34·5
,, + ,, +1·0% galactose	16·5	33

TABLE 10

Inhibition of the Respiration of Laevulose, of Glucose-6-phosphate, and of Glucose-1-phosphate* by d-mannose*

Sugar addition at 0 hrs.	O_2 uptake μl./hr./100 mg. fresh wt. −9 to 0 hrs.	+8 to +10 hrs.
No addition	18	15
0·4% laevulose	17·5	40·5
,, +0·02% mannose	18	22·5
,, +0·1% mannose	17·5	11
No addition	17·5	15·5
0·4% glucose-6-phosphate	17·5	26·5
,, +0·02% mannose	18·5	19
,, +0·1% mannose	18·5	12
No addition	16	13·5
0·4% glucose-1-phosphate	17·5	33
,, +0·02% mannose	19	22
,, +0·1% mannose	18·5	14
No addition	16·5	11·5
0·4% dextrose	18	34
,, +0·02% mannose	17	33
,, +0·1% mannose	16	31

* Concentrations shown are the hexose equivalents based on carbon content.

high rate of oxygen uptake with 3C compounds of glycolysis as substrates. Sodium pyruvate does, however, significantly stimulate oxygen uptake at least for 8 hrs. after its addition and Table 11 shows the total oxygen uptake for the 10-hr. experimental period with pyruvate and with laevulose and the effect of the addition of 0·1 per cent. mannose in each case. These results show that the mannose inhibition of respiration is not prevented by supplying as substrates the terminal or intermediate products of glycolysis.

TABLE 11

The Effect of 0·1 *per cent. d-mannose on the Oxygen Uptake of Roots Supplied with Pyruvate* (*at a concentration* ≡ *in C content* (0·4%) *to* 1 *per cent. Hexose*

Substrate(s) added at 0 hrs.	Change in the total oxygen uptake resulting from the substrate addition μl./10 hrs./100 mg. fresh wt.
No addition (control 'endogenous' respiration)	0
0·1% mannose	−48
Sodium pyruvate	+94
0·1% mannose+sodium pyruvate	7
0·4% laevulose	+121·5
0·1% mannose+0·4% laevulose	−14

DISCUSSION

The unique activity of sucrose as a source of carbon and energy for excised tomato root growth contrasts with the similar activity of sucrose, laevulose, and dextrose as substrates for respiration. Determination of R.Q. values and study of the effects of azide and DNP on oxygen uptake do not reveal any differences between sucrose and dextrose as respiratory substrates. However, the high rate of oxygen uptake established in the presence of sucrose does persist for a longer time when roots are transferred to a minus-sugar medium than is the case with roots pretreated with the monosaccharides. This suggests that some further understanding of the higher growth-promoting activity of sucrose may follow from an examination of the carbohydrate composition of roots supplied with these different actively respired sugars. The present data do not exclude the possibility that the critical factor is the rate of sucrose absorption as against monosaccharide absorption, nor do the results with azide conflict with the view that phosphorulation is involved in the mechanisms whereby these sugars reach the site of their respiration.

The view that galactose and mannose inhibit growth by different mechanisms (Ferguson, Street, and David, 1958*b*) is strongly supported by the observation that mannose does and galactose does not inhibit sucrose respiration. Those sugars which are able to reverse the mannose inhibition of root growth are the same sugars which can reverse the mannose inhibition of respiration and the concentrations required for both activities are similar. This strongly supports the view that mannose inhibits growth by its suppression of normal aerobic respiration. The inactivity of mannose-6-phosphate

as both a respiratory substrate and as an inhibitor of sucrose respiration could arise from its inability to penetrate the root cells but could alternatively indicate that mannose does not act as an inhibitor by its carbon entering the main part of carbohydrate respiration. Mannose inhibits not only sucrose and laevulose respiration (and the respiration of dextrose unless this is present in sufficient amount) but also that of some known intermediates in the Embden–Meyerhof scheme for glycolysis, including pyruvate. These observations taken together suggest that in mannose inhibition the mannose molecule itself is the active species, that it is antagonized by unmodified dextrose or xylose or galactose molecules and that the system inhibited is either in the pathway of initial absorption or of pyruvate oxidation.

Summary

'Sector' cultures of excised tomato roots maintained in a sugar-free medium for 60 hrs. yield 20-mm. root tips which have a low rate of 'endogenous' respiration. The oxygen uptake of these tips rises within the first 2 hrs. following addition to the medium of an actively respired sugar and reaches a high level within a 10-hr. experimental period.

Sucrose, dextrose, and laevulose all promote a high level of oxygen uptake. *d*-galactose, maltose, and raffinose promote a lower level of respiration; glucose phosphates are similarly active although with them the respiration rate begins to decline before the end of the 10-hr. treatment period. *d*-xylose, *d*-ribose, *l*-arabinose, sorbose, rhamnose, melibiose, lactose, dulcitol, mannitol, sorbitol, and mannose-6-phosphate are inactive as respiratory substrates. *d*-mannose inhibits the 'endogenous' respiration.

The R.Q. of root tips supplied with sucrose, dextrose, galactose, or raffinose lies within the range 0·9–0·96. The 'endogenous' respiration can have an R.Q. as low as 0·7, and in the presence of mannose as low as 0·6. These values point to carbohydrate depletion in the 'starved' tips and may indicate a complete suppression of carbohydrate respiration in the presence of mannose.

Azide, DNP, and the external pH of the medium affect the oxygen uptake similarly whether sucrose or dextrose is used as substrate. The high rate of oxygen uptake established in the presence of sucrose persists for a longer time after the removal of exogenous sugar than is the case with either dextrose or laevulose as substrates.

Dextrose, galactose, xylose, arabinose, and glucose-6-phosphate do not inhibit sucrose respiration. Mannose is strongly inhibitory at 0·02 per cent. and completely inhibitory at 0·1 per cent. to the respiration of externally supplied sucrose. Mannose is more inhibitory to the respiration of mature cells than to those of the meristematic region. Prolonged exposure to galactose does not impair the respiration of mature cells but does reduce the respiratory activity of the root meristems.

The inhibition of sucrose respiration by mannose can be fully reversed by an appropriate addition to the external medium of either dextrose or xylose, and partially reversed by addition of galactose. The respiration of laevulose,

glucose-1-phosphate, glucose-6-phosphate, and pyruvate is inhibited by mannose.

The results are discussed in relation to the growth effects of the sugars tested.

Literature Cited

Beevers, H., 1953: 2,4-Dinitrophenol and Plant Respiration. Amer. J. Bot., **40**, 91.

Dormer, K. J., and Street, H. E., 1949: The Carbohydrate Nutrition of Tomato Roots. Ann. Bot., N.S., **13**, 199.

Ferguson, J. D., Street, H. E., and David, S. B., 1958*a*: The Carbohydrate Nutrition of Tomato Roots. V. The Promotion and Inhibition of Excised Root Growth by Various Sugars and Sugar Alcohols. Ann. Bot., N.S., **22**, 513.

—— —— —— 1958*b*. The Carbohydrate Nutrition of Tomato Roots. VI. The Inhibition of Excised Root Growth by Galactose and Mannose and its Reversal by Dextrose and Xylose. Ann. Bot., N.S., **22**, 525.

Hunter, F. E., 1951. Oxidative Phosphorylation during Electron Transport, In Phosphorus Metabolism, vol. I. Baltimore: The Johns Hopkins Press.

Rothstein, A., 1954. The Enzymology of the Cell Surface. Protoplasmatologia, Handbuch der Protoplasmaforschung, Band 2, E. 4, 1–86. Springer-Verlag, Innsbruck.

Simon, E. W., 1953. Mechanism of Dinitrophenol Toxicity. Biol. Rev., **28**, 453.

Stenlid, G., 1948. The Effect of Sodium Azide on the Exudation and Oxygen Consumption of Excised Plant Roots. Physiol. Plant., **1**, 185.

Street, H. E., and Lowe, J. S., 1950: The Carbohydrate Nutrition of Tomato Roots. II. The Mechanism of Sucrose Absorption by Excised Roots. Ann. Bot., N.S., **14**, 307.

—— and McGregor, S. M., 1952: The Carbohydrate Nutrition of Tomato Roots. III. The Effects of External Sucrose Concentration on the Growth and Anatomy of Excised Roots. Ibid., **16**, 185.

White, P. R., 1943: A Handbook of Plant Tissue Culture. The Ronald Press Company, New York.

Witter, R. F., and Newcomb, E., 1952: Mechanism of Action of Dinitrophenol. Fed. Proc., **11**, 312.

Studies on Extension Growth in Coleoptile Sections

II. The Effects of High Concentrations of β-indolylacetic Acid on Section Growth

BY

C. R. HANCOCK

(*Research Institute of Plant Physiology, London, working at East Malling Research Station, Kent*)

With three figures in the Text

Abstract

Using wheat coleoptile sections it has been shown that the treatment given between cutting them from the coleoptile and placing them in the test solution greatly affects the early growth-rates.

After growing sections in high concentrations of IAA for some hours it is necessary to give a considerable number of washings to free them from surplus IAA; if these sections are then grown in water they reach lengths greater than those of comparable sections grown in water all the time, and in some cases greater than those attained in 'optimum' IAA concentrations.

There is no suggestion in the experiments described that high concentrations of IAA result in initial growth-rates higher than those observed in 'optimum' concentrations, and at very high concentrations reduced early growth-rates are indicated.

These results, and those described for lower IAA concentrations in the first paper of this series, have some bearing on the application of the enzyme kinetic theory to auxin-induced growth, and this is considered in the discussion.

Introduction

THE application of enzyme kinetics by Foster, McRae, and Bonner (1952) to coleoptile section extension has provided a useful means of analysis of the effects on growth of mixtures of growth promoters (McRae, Foster, and Bonner, 1953) and promoters and inhibitors (McRae and Bonner, 1953) and, in particular, has suggested how a highly active growth promoter such as β-indolylacetic acid (IAA) may become inhibitory at high concentrations (Foster, McRae, and Bonner, 1952). It is unfortunate that the method employed to obtain a value of 'initial growth-rate', essential to kinetic analysis, is open to criticism, especially at high concentrations of IAA (Bennet-Clark and Kefford, 1954; Bennet-Clark, 1956; Housley, Bentley, and Bickle, 1955; Marinos, 1957), since it assumes a linear relation between time and section growth over the first 12 hours. The American workers subsequently demonstrated that under the special conditions of their experiments such linearity was certainly found at concentrations of IAA up to 100 ppm. (Bonner and Foster, 1955), but was not specifically shown at the higher concentrations which they had used in their previous publication (Foster, McRae, and Bonner, 1952) to determine the constant used in their modified enzyme kinetic equation.

[Annals of Botany, N.S. Vol. 23, No. 89, 1959.]

Growth substance assays are carried out in this laboratory by the coleoptile straight growth test using wheat coleoptile sections, which although showing inhibition of growth at high concentrations of IAA, are far more resistant to the damaging effects of such solutions, and might therefore be suitable material for investigating the direct effect of 'supra-optimal' concentrations of IAA on growth. As, however, it has been shown (Barlow, Hancock, and Lacey, 1957) that under these experimental conditions a curvilinear relationship exists between coleoptile extension and time at all concentrations of IAA, it was considered essential to carry out frequent early measurements of growth in order to obtain a more direct estimate of 'initial growth-rates' at high concentrations of IAA.

In the course of this work it became abundantly clear that minor variations in handling the sections between cutting and placing in IAA solutions had considerable effects upon the early growth-rates; experiments dealing with this aspect are therefore reported first, followed by the results of experiments designed to examine claims that high concentrations of IAA prove toxic to coleoptile sections soon after being applied. Finally the implications of the results are briefly considered in connexion with kinetic analysis of section growth.

Material and Methods

In general the experiments were conducted using the routine test method previously described (Barlow, Hancock, and Lacey, 1957). Wheat seedlings are grown in sand and when at a suitable stage the coleoptiles are removed from the seed and graded for length. From each coleoptile within a given grade (range 5 mm.) a 1 cm. section is cut 3 mm. from the tip. In the routine tests sections are then placed in bundles of five on moist filter paper in petri-dishes until sufficient have been collected for the test, but in some of the experiments to be described the bundles of sections were placed directly in the solution as soon as they were cut. The five sections are placed in a small specimen tube (50 mm.$\times$9 mm.) with 0·5 ml. of solution and the tubes rotated on a klinostat. In routine tests the sections are measured to the nearest 0·5 mm., but in those experiments in which frequent measurements were made the lengths were estimated to the nearest 0·25 mm. The IAA solutions throughout are of the sodium salt made up in 'resin-filtered' distilled water. In some experiments sections were grown for a period in one solution and then transferred to a second solution. In order to remove surplus IAA solution the sections were removed from the tube with a hooked needle, shaken gently in 20 ml. water, transferred to a fresh lot of water and again shaken; this process was repeated up to eight times.

Results

The effect of storage of sections upon early growth-rates in IAA

When sections grown in a wide range of IAA concentrations were measured frequently it was found that there was considerable fluctuation in the

growth-rates during the early periods; it was suspected that this was in some way associated with the storage of the sections prior to adding them to the solution, and the effect of storage upon the growth-rates was therefore investigated. A comparison was made between the growth of sections placed in the test solution immediately after cutting and that of similar sections stored

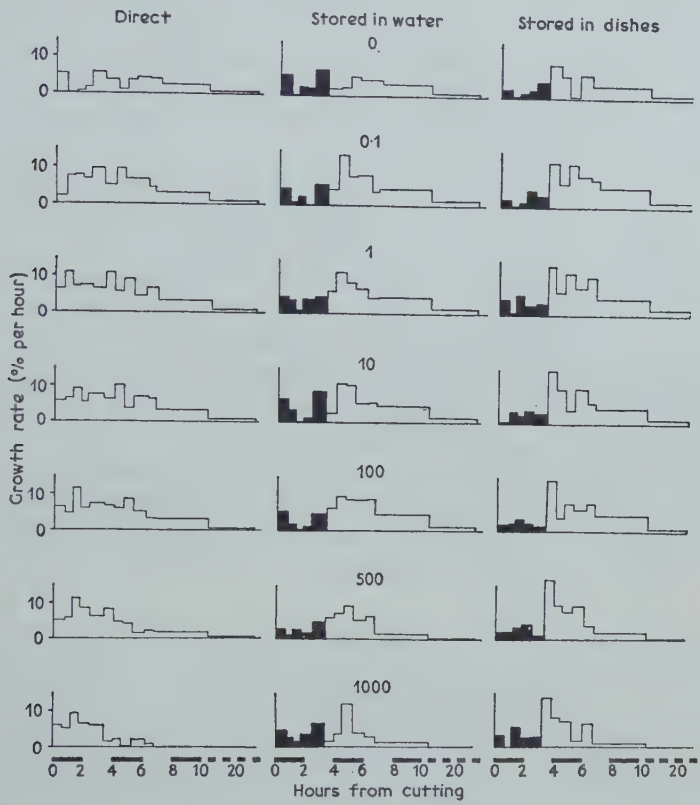


FIG. 1. Growth-rate histograms for coleoptile sections which after being cut from the coleoptile were either placed directly into IAA solution, or were stored for about 3 hours (solid histograms) in tubes of water or in moist petri-dishes before being placed in the test solutions. Concentrations of IAA are shown above each set of histograms. The time-scale has been contracted after 10 hours. To facilitate rapid comparison of the time-scales in the diagrams in this paper, 2-hourly intervals are indicated throughout by alternate black and white lines.

either in tubes of water on the klinostat or in moist petri-dishes, as in routine assays in this laboratory. The storage of sections in water may be compared with the method adopted by many workers in which sections are kept in a dish of water until a sufficient number have been acquired. The results of this experiment are presented in Fig. 1.

It will be seen that the growth-rates fluctuate considerably in successive approximately half-hourly periods, and placing the sections directly in the solution has not eliminated this variability.

Fairly high standard errors were found, suggesting that some of this fluctuation is likely to be due to the inherent inaccuracies in our present method of measuring the sections, and if valid growth-rates are to be calculated over short periods then an improved technique will be necessary by which rapid but accurate measurement of large numbers of sections can be made. Even so it is probable that fluctuations would be shown, such as those recorded by the auxanometer described by Idle (1955, 1957) and some of those seen in Fig. 1 are no doubt 'real'. Thus in the water-stored sections (Fig. 1 middle column) up to the time of transfer there are seven sets of tubes which may be grouped together, plus the 'direct' treatment in water (Fig. 1, left-hand column, top row). Considering these eight sets it is clear that in almost every case the growth is higher in the first period than in the next three periods, and then rises again to the time of transfer (average values for the five periods are: 4·66, 1·86, 1·44, 1·92, 5·98 per cent. per hour; sig. diff. 1·58, $p = 0{\cdot}01$). This fall and rise in growth-rate may represent the time taken for the section to 'use up' its auxin and then regenerate a 'physiological tip'; the duration and the degree of the fall and rise may well be associated with the 'intrinsic growth-rate' of the section (Barlow, Hancock, and Lacey, 1957) while, as these sections are grown without sucrose, the high growth-rate in the first period after cutting will include the 'water saturation' of the section described by Burström and Fransson (1957). This complex shape of the growth curve makes it virtually impossible to assign a value to the 'initial growth-rate' of these sections, and in whatever way it may be modified by exogenous auxin, it should not merely be ignored when estimating the effect on growth of an external supply.

If the growth-rates after placing the sections in IAA solutions are considered, then, in spite of the general variation between successive periods, certain overall effects of storage treatment are evident. For example, grouping all IAA concentrations together for analysis, it is found, (*a*) that in the first period in IAA solution, sections stored on moist filter paper in dishes exhibited a significantly higher growth-rate than that achieved by sections in the other two treatments either in that or in any other period (significance level throughout, $p = 0{\cdot}01$). (*b*) In the first half-hour in IAA, sections which had been stored in water in fact showed no response to the growth substance, the growth-rate being similar to, or less than, that in the period immediately before transfer, although significantly higher rates were attained in one of the next two periods. (*c*) These higher rates of growth are similar to the rate reached by sections in the third period after being placed directly in IAA (Column 1, Fig. 1), although, as mentioned above, during this period in water only, the growth-rate had fallen to a low level.

This experiment shows that estimates of initial growth-rates will be considerably affected by the treatment of the sections prior to the addition of the growth substance. The complex shape of the curve makes extrapolation to zero time, as done by Bennet-Clark and Kefford (1954), of doubtful validity, and whether attempted or not, there is no conclusive evidence from the data

presented in Fig. 1, or in similar data in which average rates over 1-hour periods are presented (Fig. 2), that the growth-rates are increased at the higher IAA concentrations, while at the highest concentrations they appear to be definitely reduced.

It is of interest that if the final response of sections is all that is required from an assay method, then sections may be stored for some hours, either in

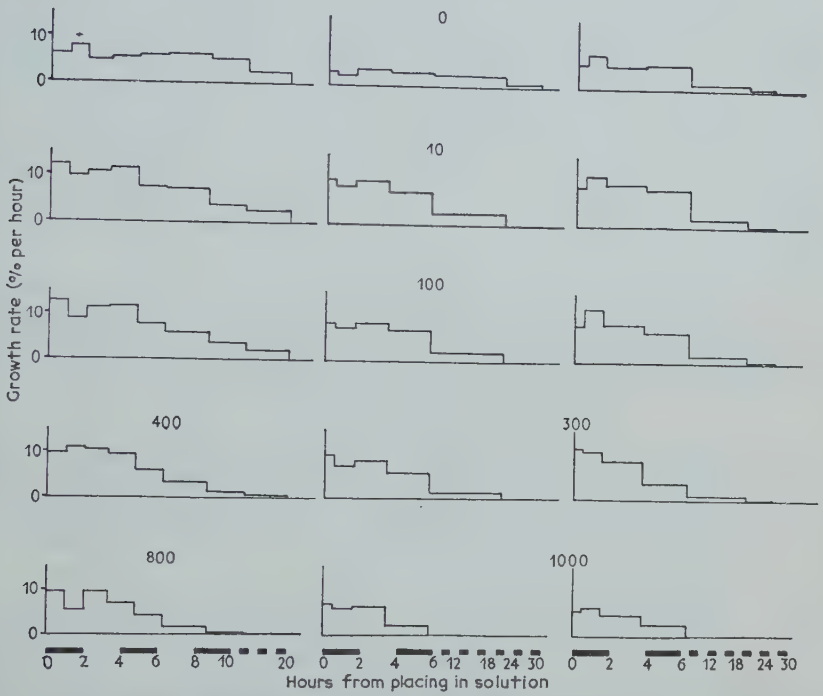


FIG. 2. Growth-rate histograms from three experiments in which coleoptile sections were grown in IAA solutions of 0, 10, 100, 400, and 800 ppm. (first column) or 0, 10, 100, 300, and 1,000 ppm. (second and third columns). 2-hourly intervals are indicated by alternate black and white lines; the later part of the time-scale has been contracted.

moist dishes or in water, without markedly affecting their growth; in this experiment, in spite of the large effects on growth-rates over limited periods, these storage régimes had no significant effect upon final length.

Regrowth of sections after treatment with high IAA concentrations

It was observed in the experiments described above that, even at the highest concentrations of IAA, the sections had not shrunk after 16 hours in the solution, although after 6 hours growth had slowed up considerably, and by 10 hours had almost ceased in concentrations above 400 ppm. In view of this it was decided to transfer sections to water after a period in high concentrations of IAA, and to observe whether they recovered or had been permanently damaged by such an exposure as Housley, Bentley, and Bickle (1954) contend.

In conducting such experiments it was thought that the tubes with transferred sections might contain dilute solutions of IAA rather than water, due to carry-over of the growth substance in, or on, those sections, particularly if they came from high IAA treatments. Sections were therefore kept in a range of IAA concentrations for 5 minutes and for 5 hours, and then both lots were washed four times as already described; the solutions produced by the first, second, and fourth rinses were then tested for activity with fresh sections.

After 5 minutes' immersion, even sections from high IAA concentrations carried over relatively little, so that by the second rinse all activity had been lost; e.g. sections placed originally in 250 and 1,000 ppm. provided second rinsings which caused growth increases of fresh sections of 64 per cent. and 62 per cent. respectively, compared with 67 per cent. for similarly treated water controls.

The results of testing the rinsing water from the sections kept for 5 hours in IAA are shown in Table 1.

TABLE 1

Percentage Increase over Original Length of Fresh Sections in Solutions Obtained by Washing 5 sections in 20 ml. of Water, 1, 2, or 4 times, after Exposure to a Range of IAA Concentrations for 5 hours

Rinse number	IAA concentration in *original* solution (ppm.)						
	0	0·98	3·9	15·6	62·5	250	1,000
1	53	59	63	72	85	95	103
2	54	56	60	64	65	78	80
4	56	55	56	60	62	64	68
Fresh IAA	55*	98	—	103	94	—	—

* Fresh IAA at 0·008 and 0·12 ppm. gave responses of 65 and 90 respectively.

After this longer period of immersion, either because growth of the coleoptile cylinder beyond the enclosed leaf provided a small space difficult to free from solution, or because of entry into the cell walls, the sections carried over an appreciable amount of IAA, and this could be detected even in the fourth rinsing from sections which had been in high concentrations.

In view of this carry-over even after four washes, in the next experiment in which the behaviour of the sections themselves was followed, additional washing was included. Sections were kept for $5\frac{1}{2}$ hours in a range of high IAA concentrations and then samples were washed either 2, 4, or 8 times before being grown on for a further period in water. Table 2 shows the final lengths of these sections (per cent. increase over original length), and of comparable ones grown all the time in IAA solutions, including some lower concentrations.

It will be seen that some of the sections grew better after being in a 'supra-optimal' concentration of IAA for a few hours than when in the 'optimum' (1·95 ppm.) all the time; this will also be noted in connexion with the experiment shown in Table 3.

Apart from this feature, the results show that the growth of sections transferred to water after high IAA treatments is not merely due to carry-over

of material on the sections, but must be due to their endogenous growth-substance content together with any exogenous promoter absorbed and retained by the sections, i.e. not removed by considerable washing.

It will also be seen that even after immersion for $5\frac{1}{2}$ hours in 1,000 ppm.

TABLE 2

Percentage Increase over Original Length of Sections Grown in IAA Solutions for $5\frac{1}{2}$ hours, and then Transferred to Water after Washing 2, 4, or 8 times in Large Volumes of Water

Number of times washed	IAA concentration in *original* solution (ppm.)					
	0	62·5	125	250	500	1,000
2	68	116	112	102	100	89
4	64	115	111	107	102	90
8	71	107	106	103	92	80

Sections not transferred	IAA concentrations (ppm.)						
	0	0·008	0·12	1·95	7·8	250	1,000
	67	76	101	105	102	80	32

TABLE 3

Growth-rates During the First 8 hours (up to Time of Transfer), and in Two Later 12-hour Periods, of Sections in IAA Solution as per cent. of Rates in Water. (a) *Remaining in Original Solution and* (b) *Transferred to Water.*

IAA	Periods (hours from start)					
	0–8		8–20		20–32	
ppm.	*a*	*b*	*a*	*b*	*a*	*b*
0·12	286	300	105	75	33	33
0·24	314	336	110	60	40	56
0·98	314	314	110	95	67	33
3·9	300	314	105	120	73	44
15·6	306	314	105	160	87	111
62·5	294	314	80	160	53	145
125	294	328	75	145	60	145
500	240	228	32	145	67	167
1,000	173	193	13	140	67	144

IAA, sections have made a large amount of growth; the fall in final length as the preliminary treatment concentrations rise from 62·5 to 1,000 ppm. is accounted for by the effect of these solutions on the section during the treatment period, rather than by permanent damage—the growth-rates rose after release from the inhibitory effect of high IAA concentrations, but the initial setback could not be compensated for completely.

The results of a similar experiment (in which unfortunately the sections were only washed twice, so that they may in fact have been growing after transfer in dilute IAA and not water) are shown in Fig. 3. Here the sections

were grown for 8 hours in a range of IAA solutions, at which time half were washed and transferred to water, and the other half left to continue extension in the same solutions. It will be seen that at the higher concentrations (left-hand diagram) the growth-rates of the sections left in the solutions (solid symbols) fell off after 8 hours, but those transferred to water (open symbols) continued to grow at a high rate; at intermediate concentrations (top curve of right-hand diagram) there is virtually no effect of transfer, while at low

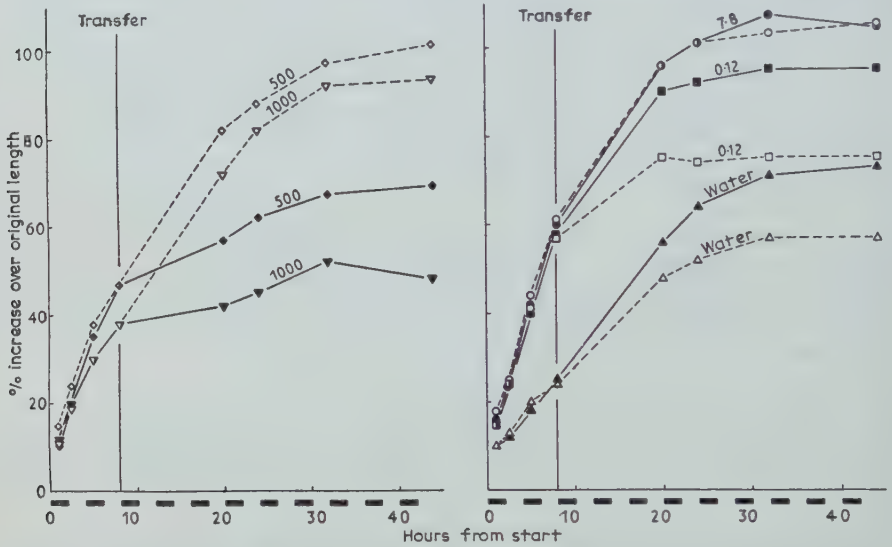


FIG. 3. Growth curves for sections grown for 8 hours in water or IAA solutions of the concentrations shown on the graphs, and then either left in the same solution for a further 36 hours (solid symbols), or transferred to water (open symbols).

concentrations the growth was reduced by transfer to water. Part of this effect was due to transfer *per se*, as shown for water (lowest pair of curves on the right-hand diagram where carrying out the same procedure in water reduced the growth-rates somewhat). For this reason in Table 3, which shows the growth-rates for further concentrations from the complete range studied, they have been expressed as relative to the rates in water for (*a*) 'left in the solution' and (*b*) 'transferred to water after washing'. Average rates for three periods are shown: Up to time of transfer (0–8 hours from start), and two subsequent 12-hour periods (8–20 hours and 20–32 hours from start).

It will be seen that the values in the first two columns agree reasonably well, these being the rates for the two sets of tubes during the 8 hours before transfer, while they were of course being similarly treated; growth is markedly reduced at concentrations above 125 ppm.

In the second period all rates were lower, but those for the two sets diverged; the growth-rates of sections left in the original solutions fell steadily as the concentration was increased above 0·98 ppm., whereas the rates of sections

transferred to water were high, even after having been in supra-optimal concentrations as great as 1,000 ppm.; the same holds true in the third period.

As already mentioned in connexion with Table 2 the sections which were transferred from high concentrations of IAA to water in fact showed higher growth-rates than those left in the 'optimum' concentration all the time, in spite of the slightly deleterious effects of transfer as such; in any case there is no suggestion that these high concentrations have caused permanent damage to the section, although growth had been checked while the solution was in contact with the sections.

Discussion

The main points brought out by the experiments described in this paper are as follows:

(*a*) High concentrations of IAA reduce the total growth made by the sections due to a shorter period of growth, a more rapid decline of the growth-rate, and, at the highest concentrations employed, a reduction in the earliest observed growth-rates. (*b*) Sections grown for 5 hours in IAA solutions are very difficult to free from IAA which can be detected even after washing the sections four times in large volumes of water. (*c*) Sections which have been in high concentrations of IAA and which have been freed of excess by washing up to 8 times in water will, when subsequently grown in water, increase in length as much as, and sometimes more than, similar sections grown in the 'optimum' IAA all the time. (*d*) The early growth-rates of coleoptile sections may be affected very greatly by the treatment of the sections between the time of cutting them from the coleoptile and that of adding the growth substance solution, even though there is little effect upon final length.

Since these experiments were carried out, a paper has appeared (Marinos, 1957) in which the results of some similar experiments have been described, and their bearing on the application of the enzyme kinetics equation to coleoptile section growth discussed. The experiments described by Marinos were carried out with oat coleoptile sections, and he experienced similar difficulties to those of other workers who have investigated the effects of high concentrations of IAA on section growth: namely, that there is a very rapid fall in growth-rate followed by visible damage and shrinkage of the sections. The present work has shown, however, that if wheat coleoptile sections are used, it is possible to obtain continued growth at concentrations of IAA up to 1,000 ppm. for considerable periods without visible damage, although, as with oat coleoptile sections, the optimum growth (as judged by final unshrunk length) takes place at about 10 ppm. It has been shown that at these supra-optimal concentrations there is no permanent damage to the sections for at least the first 8 hours of their growth, since such sections after being freed of surplus IAA will continue growth in water as well as sections kept in optimum IAA all the time. This is in contrast to the results of Marinos, who confirms

the claims made by Housley, Bentley, and Bickle (1954), and the contention of Bennet-Clark (1956), that the lower early growth-rates obtained in high concentrations of IAA can be explained by secondary effects due to destruction of part of the cell mechanism. He has shown (Marinos, 1957) that oat sections taken from a concentration of 180 ppm. and transferred to water after only 1 hour, do not grow as well as those in optimum IAA, and if left in the high concentrations for 2½ hours grow hardly at all when placed in water.

The early growth-rates in concentrations at and somewhat above the 'optimum' (1–100 ppm.) may well all be similar in the experiments described here, and, indeed, in some carried out later at low temperatures (to be described in a subsequent paper in this series) there were some indications that early growth in 100 ppm. was faster than in that concentration giving the maximum final length. In all experiments, however, at higher concentrations (e.g. Fig. 1, 1,000 ppm., and Fig. 2, 800 and 1,000 ppm.) the earliest observed growth-rates were definitely reduced. Marinos presents figures showing that although in supra-optimal concentrations of IAA and other auxins damage to the sections occurs fairly early, during the first half-hour they attain a length considerably in excess of that shown in optimal concentrations; only at 1,800 ppm. IAA was the half-hour measurement below the optimum. Bennet-Clark and Kefford (1954) also claim higher initial growth-rates at supra-optimal concentrations, their method of evaluation at high IAA concentrations being by extrapolation back to zero time on growth-rate histograms. It would appear to the present author, however, that any method of extrapolation used should be carried out at all concentrations, i.e. low as well as high, and, as has already been mentioned, this is virtually impossible when the growth-rate increases for some time before reaching its maximum rate as is the case in low IAA concentrations both in the results shown by Bennet-Clark and Kefford, and those in the previous paper of this series (Barlow, Hancock, and Lacey, 1957).

The experiments in which coleoptile sections were stored under various conditions show that a further complication may occur in ascertaining an 'initial growth-rate': if, for instance, the sections are stored in moist dishes, a very high rate of apparent 'growth' occurs during the first period after placing the sections in the solution; this may well be compared with the results obtained by Cleland and Bonner (1956) where the effect of added auxin has been separated from the subsequent cellular extension by water uptake. In our case the endogenous auxin may perhaps be acting upon the cell wall during dish storage where absence of free water does not permit expansion; when the sections are subsequently placed in water or IAA this expansion takes place, and for a short time a high growth-rate results.

Apart from this special case of dish storage affecting early growth of the section, however, there is the more general difficulty of allowing in some way for the endogenous auxin. The growth of sections in water may, when analysed in detail, sometimes follow a very complex course (as in Fig. 1), with a falling rate for a short time followed by a rise before the eventual decline as the

section reaches its final length. The first period of relatively high growth-rate could perhaps be accounted for by the 'water saturation' of the section described by Burström and Fransson (1957), but the subsequent rise and fall which is similar to the trends shown in water and low concentrations in the first paper of this series (Barlow, Hancock, and Lacey, 1957) and by Bennet-Clark and Kefford (1954), implies a changing supply of endogenous auxin (or at least a changing response of the cell). This situation complicates the assessment of the effects of exogenous auxin at concentrations too low to dominate growth, where the endogenous supply plays an important part. The effects of these low concentrations, therefore, have been given less weight by McRae *et al.* (1953) in their calculations of kinetic constants.

The question of the part played by the endogenous auxin has been given some consideration by Kaindl (1956) who, when calculating his action-concentration curves of growth substances, considered the endogenous and exogenous promoters as possibly competing for growth sites in the living system. It would seem that over the lower part of the concentration range the exogenous and the endogenous promoters might well be treated by the methods outlined by McRae *et al.* (1953) for mixtures of synthetic growth substances. This would lead to a more logical consideration of growth of the section in water, i.e. due to the endogenous auxin alone, a point which would appear to have been curiously neglected so far. The British and American workers have either completely ignored the growth in water, or merely deducted it from that in the presence of added growth substance, while, on the other hand, Ingestad (1953) calculated the concentration of the endogenous auxin in terms of the exogenous promoter and added these together.

It is realized that there are considerable practical difficulties which may preclude the evaluation of the requisite constants for the endogenous auxin, but the approach may be briefly considered. In treating the endogenous and exogenous auxins as a mixture of growth substances, the concentration of endogenous auxin can be taken as constant at a particular time within a given experiment. At high concentrations of exogenous auxin the molar ratio will be so much in favour of the applied substance that the theoretical relationship between growth-rate and exogenous auxin concentration may hold; as its concentration is reduced, however, so the effect of the endogenous auxin plays a greater part, and depending on the affinities of these two substances for the receptor sites so the observed effect of the exogenous promoter will become greater or less than predicted. If under particular circumstances the endogenous auxin is reduced to a low level (Bonner and Foster, 1955) then the added auxin will continue to dominate growth at lower concentrations and the theoretical relationship may hold over a wider range of dilutions, and only at very low concentrations of applied auxin will the competitive effect appear. At very low concentrations of applied promoter growth less than that in water controls has been observed (Barlow *et al.* 1957), which is a very similar condition to that described by Bonner (1956) for the interaction at low concentrations of IAA and some second auxin, when the latter inhibits the

growth-promoting effect of IAA, a situation which he is unable to interpret by the simple kinetic theory.

It would appear that the concept of initial growth-rate cannot be applied to so complex a system as a coleoptile section, since, unlike the combinations of an enzyme system with its substrate, which starts from zero velocity until the two are combined, the coleoptile section has a growth-rate at the time it is taken from the seedling which varies with the age of the coleoptile from which it is cut, and the time elapsing before the addition of the growth substance. Furthermore, the transport of the applied growth substance to the site of action must also be considered (Housley, Bentley, and Bickle, 1954). These complicating factors must be allowed for in any adequate kinetic analysis aimed at relating the concentration of applied growth substance with growth; it may then be possible to use such a method of analysis to elucidate growth reactions under much less restricted conditions than have in fact been previously used.

Summary

1. Considerable fluctuations in growth-rates are observed when wheat coleoptile sections are measured frequently during the course of their growth in water and IAA solutions.

2. Storing the sections in moist petri-dishes or in water for about 3 hours, or placing them directly in the test solution, may greatly affect these fluctuations, particularly during the early growth, but has no effect upon the final length.

3. Sections which have been grown for 5 hours in high concentrations of IAA and then rinsed in successive 20 ml. lots of water, continue to provide detectable amounts of auxin in at least four such washings.

4. High concentrations of IAA (up to 1,000 ppm.) cause no permanent damage to wheat sections for some hours. Sections which have been in supra-optimal concentrations and then washed free of excess growth substance, will grow in water considerably more than those in water all the time, and in some cases reach lengths greater than those attained by sections in 'optimum' concentrations of IAA throughout their growth.

5. There is no suggestion in the experiments described that high concentrations of IAA result in higher initial growth-rates than those in 'optimal' concentrations, and at very high concentrations reduced initial growth rates are indicated.

6. These results, and those described in the first paper of this series, are considered in connexion with the application of the enzyme kinetic theory to auxin-induced growth.

Literature Cited

Barlow, H. W. B., Hancock, C. R., and Lacey, H. J., 1957: Studies on Extension Growth in Coleoptile Sections. I. The Influence of Age of Coleoptile upon the Response of Sections to IAA. Ann. Bot., N.S., **21**, 257–71.

Bennet-Clark, T. A., and Kefford, N. P., 1954: The Extension Growth-Time Relationship for *Avena* Coleoptile Sections. J. exp. Bot., **5**, 293–304.

Bennet-Clark, T. A., 1956: The Kinetics of Auxin-induced Growth. The Chemistry and Mode of Action of Plant Growth Substances. Butterworth, London.

Bonner, J., and Foster, R. J., 1955: The Growth-time Relationships of the Auxin-induced Growth in *Avena* Coleoptile Sections. J. exp. Bot., **6**, 293–302.

—— 1956: The Kinetics of Auxin-induced Growth. The Chemistry and Mode of Action of Plant Growth Substances. Butterworth, London.

Burström, H., and Fransson, P., 1957: The Influence of Auxin on Water Saturated Coleoptiles. Physiol. Plant., **10**, 774–80.

Cleland, R., and Bonner, J., 1956: The Residual Effect of Auxin on the Cell Wall. Plant Physiol., **31**, 350–4.

Foster, R. J., McRae, D. H., and Bonner, J., 1952: Auxin-induced Growth Inhibition a Natural Consequence of Two-point Attachment. Proc. Nat. Acad. Sci., **38**, 1013–22.

Housley, S., Bentley, J. A., and Bickle, A. S., 1954: Studies on Plant Growth Hormones. III. Application of Enzyme Reaction Kinetics to Cell Elongation in the *Avena* Coleoptile. J. exp. Bot., **5**, 373–88.

Idle, D. B., 1955: An Auxanometer for Isolated Plant Sections. Nature, **176**, 778–80.

—— 1957: Studies in Extension Growth II. The Light-growth Responses of *Vicia faba* L. J. exp. Bot., **8**, 127–38.

Ingestad, T., 1953: Kinetic Aspects on the Growth-regulating Effect of Some Phenoxy Acids. Physiol. Plant., **6**, 796–803.

Kaindl, K., 1956: The Action-concentration Curves of Mixtures of Growth-promoting and Growth-inhibiting Substances. The Chemistry and Mode of Action of Plant Growth Substances. Butterworth, London.

McRae, D. H., Foster, R. J., and Bonner, J., 1953: Kinetics of Auxin Interaction. Plant Physiol., **28**, 343–55.

—— and Bonner, J., 1953: Chemical Structure and Antiauxin Activity. Physiol. Plant., **6**, 485–509.

Marinos, N. G., 1957: Responses of *Avena* Coleoptile Sections to High Concentrations of Auxin. Aust. J. Biol. Sci., **10**, 147–63.

Leaf Development in *Narcissus pseudonarcissus* L. I. The Stem Apex

BY

M. P. DENNE[1]

(*University College, London*)

With nine Figures in the Text

Abstract

A description is given of the daffodil apex, and of the changes in its size and organization during a plastochron and during the growing season.

The periodical production of scale and foliage leaves does not appear to correspond to seasonal changes in either the size or the organization of the apex.

Introduction

THE daffodil plant produces two types of leaf, the foliage leaves, and the scales. The aim of this investigation was to find out how these two types of leaf differ, and to discover when and how their developments diverge. Since the form of any growing object depends partly on its original form, the difference between scale and foliage leaves might be derived from a difference between their primordia. Such a change in the primordia might be due to a change in the state of the apex producing them; hence the regular seasonal sequence of leaf production might reflect a cyclical change in the state of the apex. For this reason, seasonal variations in the apex have been investigated, and are described in this paper. The further development of the leaves will be described in a subsequent paper.

Changes in the state of the apex have already been described in several plants. Abbe, Phinney, and Baer (1951) measured the apex of *Zea*, describing a change in the apical size occurring during the plastochron, and an overall change in apical size during ontogeny. Abbe, Randolph, and Einset (1941) had previously correlated the relative width of the foliage leaves of *Zea* with the relative size of the apex from plastochron to plastochron. Allsopp (1953), working with the fern *Marsilea*, related changes in the size of the apex to the heteroblastic development of the leaves. But in *Acer pseudoplatanus* White (1955) found no evidence for any regular increase in the size of seedling apices from plastochrons six to fourteen. A change in the state of the apex between the production of scale and foliage leaves has been described in *Torreya* by Kemp (1943), in *Pseudotsuga* by Sterling (1946), and in *Drimys* by Gifford (1950).

[1] Now at East Malling Research Station, near Maidstone, Kent.

[Annals of Botany, N.S. Vol. 23, No. 89, 1959.]

Materials and Methods

Narcissus pseudonarcissus var. 'Helios' has been used throughout. The bulbs were uniform, of medium size, and all produced flowers when planted in the garden. Two hundred bulbs were planted in November 1954, and 15 to 20 bulbs were dug up at monthly intervals until October 1955. The apices were fixed in chromacetic acid, and preserved in 70 per cent. methylated spirits. The apices were sectioned at 8 μ, and stained with Heidenhain's Haematoxylin and Orange G.

The Structure of the Daffodil Apex

The apex is organized into a number of zones, which are distinguished by the cytological appearance of their cells and by the direction in which new cell walls are formed.

The outer part of the apex is built up of a number of concentrically arranged layers (Figs. 1, 3); these layers arise because of a general tendency towards anticlinal cell division (i.e. new walls are formed at right angles to the surface of the apex). This tendency is most marked in the outermost layer, which divides exclusively in this direction. Towards the centre of the apex the tendency is less well marked, and in the centre the cells divide in all directions. The outermost layers, dividing exclusively or mainly anticlinally, are here loosely termed the tunica (Figs. 2, 4), while the inner region, which may be more or less arranged in layers, is termed the corpus (Figs. 2, 4). These terms cannot be more strictly defined in the daffodil, as there is a gradual difference between the two tissues, rather than a definite boundary.

The corpus region can be subdivided into three zones. There is a large group of corpus initials (Figs. 1–4); these cells are relatively large, often somewhat vacuolated. The cells of the other two zones are derived from these initials. On the side of the apex where the next primordium will arise, the derivatives of the corpus initials become more densely protoplasmic, and divide in all directions; this is the flank meristem. This is shown in Figs. 1 and 2, which illustrate an apex just before the initiation of a primordium; Figs. 3 and 4 show an apex immediately after a primordium has been initiated. On the opposite side of the apex there is a zone where the derivatives are more vacuolated, and divide to form layers of cells parallel to the tunica layers; this is the rib meristem (Figs. 1–4).

The tunica has no distinct group of initials, although the cells towards the tip of the apex are more vacuolated than those on the sides.

This zonation of the apex is similar to that reported by Stant (1954). Stant described the apices of several monocotyledons, including the daffodil, and showed that they are all constructed on the same basic pattern. A similar pattern has been described in dicotyledons and gymnosperms (e.g. Popham, 1951). But all the apices described differ from the daffodil apex in that the flank meristem is said to be cylindrical, encircling the central rib meristem, while in the daffodil the flank is restricted to the side of the apex where the

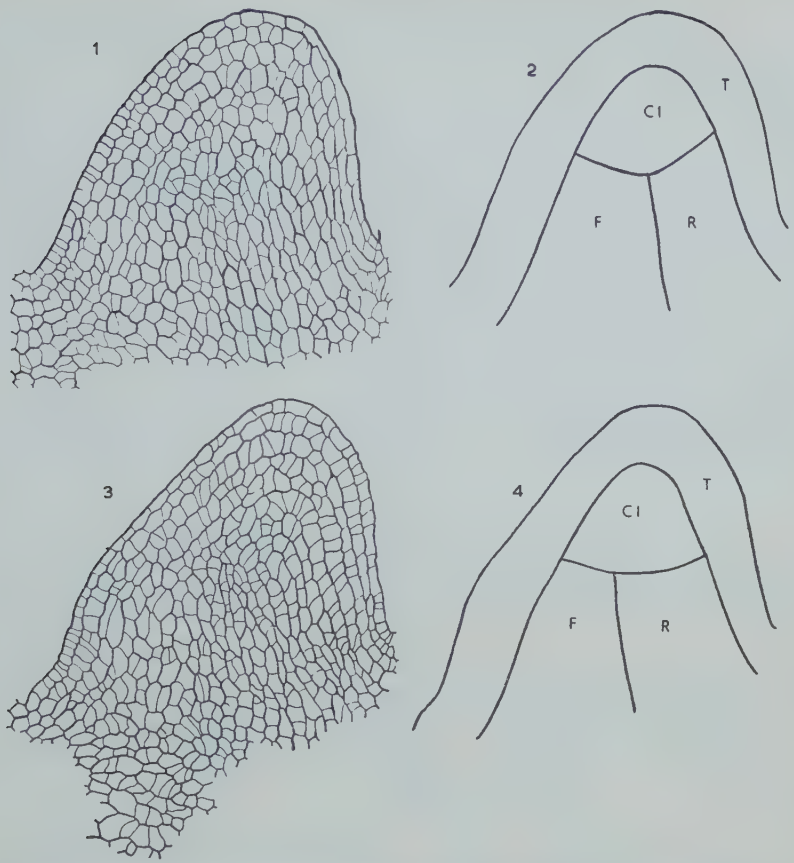


Fig. 1. Camera lucida drawing of a longitudinal section through the main apex of a daffodil bulb, cut through the median plane of the primordia. Apex in plastochron stage 3.
The next primordium is about to be initiated on the left side.

Fig. 2. Plan of the apex drawn in Fig. 1, showing the limits of the zones of the apex. Since the zones grade into each other, the limits can only be drawn approximately.
T. tunica; CI. corpus initials; R. rib meristem; F. flank meristem. CI, F, and R, together form the corpus.

Fig. 3. Camera lucida drawing of a longitudinal section through the main apex of a daffodil bulb, cut through the median plane of the primordia. Apex in plastochron stage 1.
A primordium has been initiated on the left side.

Fig. 4. Plan of the apex drawn in Fig. 3, showing the limits of the zones of the apex. Since the zones grade into each other, the limits can only be drawn approximately.
T. tunica; CI. corpus initials; R. rib meristem; F. flank meristem. CI, F, and R, together form the corpus.

next primordium is about to be formed (Fig. 2). When leaf initiation has occurred, the flank meristem extends in both directions round the apex, as cells previously behaving as rib meristem then divide in all planes to form flank meristem. Thus in the course of time the flank meristem does encircle the apex, but it is not cylindrical at any one moment. Following the course of the flank meristem the primordium slowly extends round the apex,

eventually forming a complete ring (Fig. 6). By this time the next primordium has been initiated.

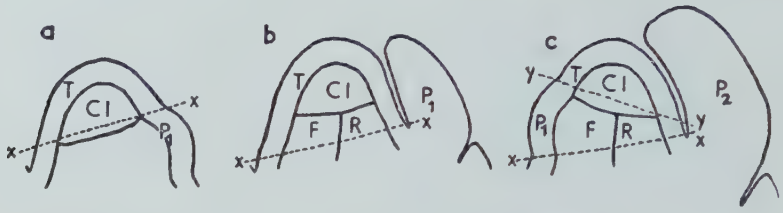


FIG. 5. Change in organization of the apex during the plastochron.

a. Plastochron stage 1.
b. Plastochron stage 3.
c. Plastochron stage 1, in the following plastochron.

CI. corpus initials; F. flank meristem; P_1 youngest primordium; P_2 second primordium; R. rib meristem; T. tunica.

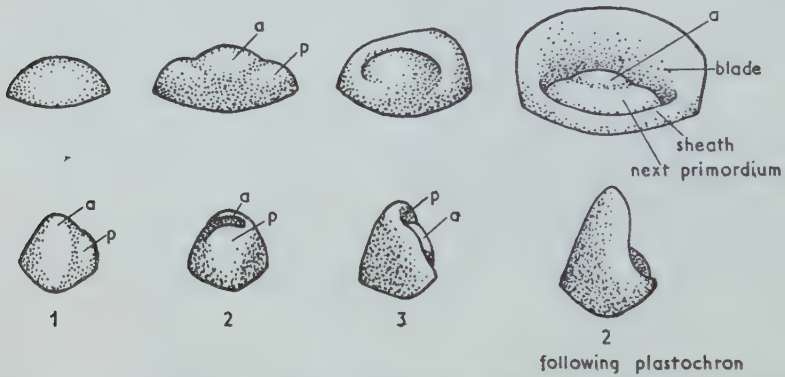


FIG. 6. Drawings of a series of apices to show the development of a primordium. The numbers along the bottom indicate the stage of the plastochron. The upper row of drawings show the ventral view of the primordium, the lower row shows the lateral view.

a. apex. *p*. primordium.

PLASTOCHRONAL CHANGES IN THE DAFFODIL APEX

For convenience in the description and measurement the plastochron can be recognized as falling into three stages.

Stage 1. Early plastochron: The youngest primordium is visible as a slight bulge. The sheath of the previous primordium does not completely encircle the apex. The apex above the youngest primordium is at its minimum volume.

Stage 2. Mid plastochron: The youngest primordium is a definite bulge, shorter than the apex. The sheath of the previous primordium almost or completely encircles the apex.

Stage 3. Late plastochron: The youngest primordium is as long as or longer than the apex. The sheath of the previous primordium encircles the apex. The apex above the youngest primordium is at its maximum volume.

Change in Organization during the Plastochron

During the plastochron there is a change in the relative proportions of the zones of the apex, as is shown in Fig. 5. In plastochron stage 1 the apex above the youngest primordium (i.e. above line XX in Fig. 5*a*) consists only of corpus initials and tunica. In plastochron stages 2 and 3 there is an increasing proportion of rib and flank meristem, as the lower corpus initials differentiate into cells of these zones, and the cells of the zones are themselves dividing rapidly. When the next primordium is produced (i.e. above line YY in Fig. 5*c*) the apex is reduced once more to corpus initials and tunica.

In the daffodil apex there is no clear distinction between the corpus initials and the overlying tunica layers. Taking these two zones together, an approximate estimate of their extent was obtained by measuring their combined depth in median radial longitudinal sections of the apex; these measurements are shown in Table 1 below.

Table 1

Plastochron stage	1	2	3
Mean depth of tunica plus corpus initials .	141·5 μ	149·5 μ	144·2 μ
Twice standard error	$\pm$5·8 μ	$\pm$4·2 μ	$\pm$4·0 μ
Number of apices measured . . .	17	14	17

These measurements were confined to the main apex of each bulb. The figures suggest that the extent of the zone of initials remains roughly constant in the course of the plastochron.

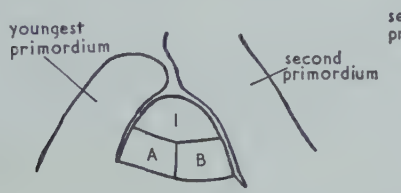


Fig. 7

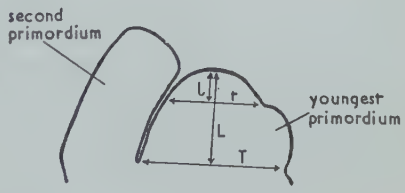


Fig. 8

Fig. 7. Diagram of a longitudinal section of an apex, cut through the median plane of the primordia, showing the regions of the apex in which cell division was measured.

Fig. 8. Diagram of a longitudinal section of an apex, cut through the median plane of the primordia.

l. minimum and L. maximum length of apex.

t. minimum and T. maximum thickness of apex.

The distribution of cell division in the apex was also measured; the relative number of dividing cells was measured in the regions shown in Fig. 7. Each region corresponds to one of the three zones of the corpus together with its overlying tunica layers. Region I includes the corpus initials. Region A lies next to the youngest primordium, and consists of rib meristem and adjacent tunica layers. Region B is on the opposite side, and consists of the flank meristem and overlying tunica layers. In this way the apex is divided into regions which are internally more or less cytologically uniform.

The apices used in this way were all in plastochron stage 3, i.e. just before leaf initiation. Sections were cut in the median longitudinal plane of the primordia. The nuclei were counted in alternate sections, as most of them could be seen in two successive 8μ sections. The rate of cell division is expressed as the number of dividing nuclei per hundred nuclei counted. This method of measuring the rate of cell division depends upon the unverified hypothesis that the relation between the duration of nuclear division and the duration of interphase is the same in all regions of the apex. At least 500 nuclei were counted in each region of each apex.

TABLE 2

	Percentage of dividing cells		
Apex No.	Region I	Region A	Region B
d52b	4·70	4·81	5·94
d53a	0·88	1·73	3·66
d57b	0·16	0·35	1·15
d59a	0·18	0·36	1·29
b138a	2·05	2·80	6·78
b139b	3·27	3·12	3·13
b141a	1·69	3·23	5·64
b144b	0·81	4·12	7·76
Mean	1·716	2·565	4·675
Approximate ratio	2 :	3 :	6

The rate of cell division in the various regions of the apex is shown in Table 2. Immediately before leaf initiation begins, the number of cells dividing in the region where the next primordium will be initiated (region B) is about twice that on the opposite side (region A). Such a situation must lead to the outgrowth of the apex on its more active side, assuming cell expansion to be about the same on both sides.

Change in Size of the Apex during the Plastochron

The size of the apex was measured from radial longitudinal sections through the median plane of the primordia. Theoretically the minimum size of the apex should be measured as soon as a primordium is initiated, but as it is impossible to define the lower edge of the apex at this stage, the minimum size was actually measured above the youngest primordium in plastochron stages late 1 to early 2, as shown in Fig. 8. The maximum length and thickness were measured below the youngest primordium at the same stage. The radial longitudinal area of the apex was calculated from the formula:

$$0{\cdot}7854(\textit{length} \times \textit{thickness}),$$

assuming the area to be approximately that of half an ellipse.

These measurements are summarized in Fig. 9, which shows that the radial longitudinal area of the apex is about doubled in the course of a single plastochron.

Seasonal Changes in the State of the Apex

The daffodil bulb grows sympodially; each year the main axis of the bulb originates from an apex in the axil of the penultimate leaf of the previous year's axis. In the season 1954–5, the first two to five primordia produced by this new main apex became scales, which were initiated in late summer 1954. These scales were followed by one or more foliage leaves in autumn 1954, and the rest of the foliage leaves in spring 1955. Finally, the main apices developed into floral apices.

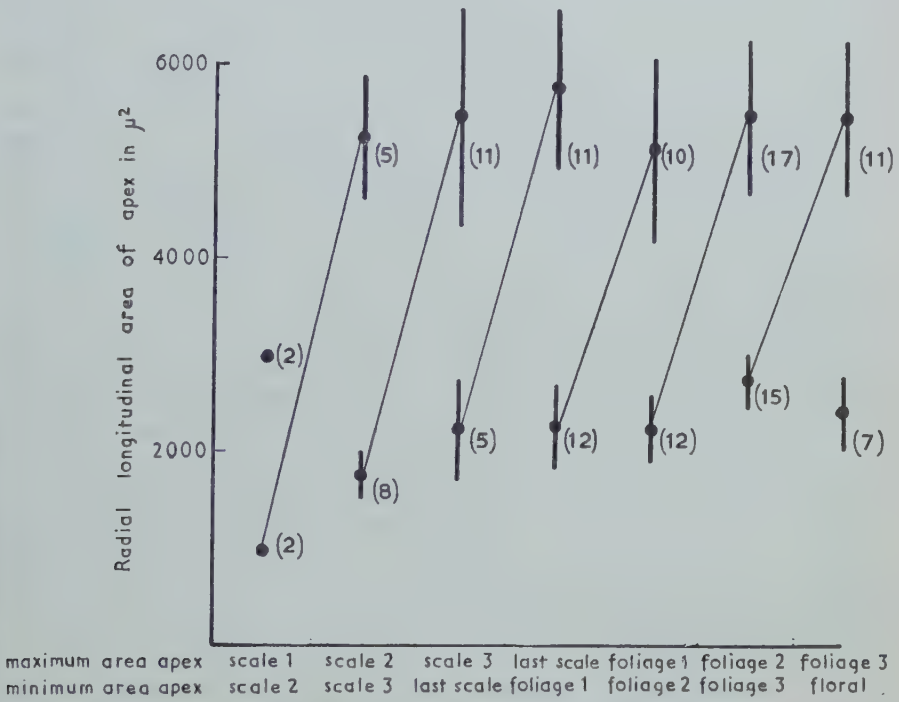


Fig. 9. The change in radial longitudinal area of the apex during successive plastochrons. The vertical lines show twice the standard error on either side of the mean. The figures in brackets indicate the number of apices measured.

The size of the daffodil apex increases rapidly from its origin as an auxillary bud until the initiation of its second or third primordium (apart from the variations during the plastochron). Fig. 9 suggests that there is no further change in size until flower initiation. White (1955) reported a similar situation in *Acer pseudoplatanus*, an increase in the size of the apex of axillary buds, but no regular increase from plastochron 6 to plastochron 14 in the size of seedling apices. Abbe, Phinney, and Baer (1951), on the other hand, found a regular increase in the size of the apex of *Zea* from plastochrons 7 to 14, and Sterling (1946) described a change in the size of the apices of *Pseudotsuga* between the initiation of scale and foliage leaves.

No seasonal changes were seen in the visible organization of the vegetative apex of the daffodil. Again this differs from the situation in *Pseudotsuga*, where Sterling (1946) found a change in the proportions of the zones of the apex between the initiation of the scale and the foliage leaves.

Discussion

It was thought that the seasonal change in the type of leaf produced might be related to a seasonal change in the size or organization of the apex. In the flowering daffodil bulb each year's main axis originates from a lateral apex. This apex increases in size after its inception. As the first few primordia to be produced on this apex are usually scales, it appeared possible that the production of scales might be related to the small size of the axillary apex. But while up to five scales are normally produced, there is no evidence of a marked change in apex size after the initiation of the second primordium. Also, bulbs are occasionally found in which one or more foliage leaves were produced on the axillary apex before the scales. Hence the size of the apex does not appear directly to influence the production of scale or foliage leaves in the daffodil.

This conclusion does not necessarily conflict with the relation of apex size to blade width described by Abbe, Randolph, and Einset (1941) in *Zea*. In the daffodil there is a noticeable difference between the blade width of foliage leaves from main and lateral shoots; possibly this is due to a difference in the sizes of the main and lateral apices. Obviously, the form of a scale or the form of a foliage leaf may be influenced by factors which do not determine the production of the one or the other type of leaf. The difference between various types of leaf may be merely an exaggeration of different forms of one type, but not necessarily so; in *Marsilea*, Allsopp (1955) brought about the production of air or water types of leaf by submerging the plant in media of different osmotic pressures, while changes in the form of one type of leaf were produced by altering the nutrient status of the medium (Allsopp, 1953).

Buvat (1952) and his co-workers have described a zonation of the apex based on the distribution of mitotic activity. They describe the tip of the apex as a *méristème d'attente*, a region in which there are no cell divisions while the plant is vegetative. In the daffodil apex there are fewer cell divisions at the tip (Table 2, region I), but divisions were frequently seen, even at the extreme tip. Below the *méristème d'attente* Buvat described a meristematic ring, the *anneau initial*, from which the primordia are initiated. This *anneau initial* may be compared with region B, a rapidly dividing region consisting of the flank meristem and its overlying tunica; though, as is described above, the flank meristem encircles the apex in the course of a plastochron, it is not a ring at any particular time. The position of this centre of active growth may be determined by the position of the older primordia; region B is farther from the nearest primordium than is region A, so if cell division is limited by the lack of materials used up by this primordium, or directly inhibited by the chemical or physical conditions near the primordium, then cells in region B would be expected to divide more frequently than cells in region A.

Acknowledgements

I would like to express my thanks to my Supervisor, Dr. D. J. B. White, for all his help and good advice, to Professor W. H. Pearsall for many useful discussions, and to the Department of Scientific and Industrial Research for a maintenance grant. The material in this paper is part of a thesis approved for the degree of Ph.D. in the University of London.

Literature Cited

Abbe, E. C., Phinney, B. O., and Baer, D. F., 1951: Growth of the Shoot Apex in Maize; Internal Features. Amer. J. Bot., **38**, 744.

—— Randolph, L. F., and Einset, J., 1941: The Developmental Relationship between Shoot Apex and Growth Pattern of Leaf Blade in Diploid Maize. Ibid., **28**, 778.

Allsopp, A., 1953: Experimental and Analytical Studies of Pteridophytes. XX. Investigations on *Marsilea*. 3. The Effect of Various Sugars on Development and Morphology. Ann. Bot., n.s., **17**, 447.

—— 1955: Experimental and Analytical Studies of Pteridophytes. XXVII. Investigations on *Marsilea*. 5. Cultural Conditions and Morphogenesis, with Special Reference to the Origin of Land and Water Forms. Ibid., **19**, 247.

Buvat, R., 1952: Structure, évolution et fonctionnement du méristème apical de quelques dicotyledones. Ann. Sci. Nat. Bot., Ser. 11, **13**, 199.

Gifford, E. M., 1950: Structure and Growth of the Shoot Apex in Certain Woody Ranales. Amer. J. Bot., **37**, 595.

Kemp, M., 1943: Morphological and Ontogenetic Studies on *Torreya californica* Torr. I. The Vegetative Apex of the Megasporangiate Tree. Amer. J. Bot., **30**, 504.

Popham, R. A., 1951: Principal Types of Vegetative Shoot Apex Organization in Vascular Plants. Ohio Journ. Sci., **51**, 249.

Stant, M. Y., 1954: The Shoot Apex of some Monocotyledons. II. Growth Organization. Ann. Bot., n.s., **18**, 441.

Sterling, C., 1946: Organization of the Shoot of *Pseudotsuga taxifolia* (Lamb.) Britt. I. Structure of the Shoot Apex. Amer. J. Bot., **33**, 742.

White, D. J. B., 1955: The Architecture of the Stem Apex and the Origin and Development of the Axillary Buds in Seedlings of *Acer pseudoplatanus* L. Ann. Bot., n.s., **19**, 437.

Physiological and Ecological Studies in the Analysis of Plant Environment

XII. The Role of the Light Factor in Limiting Growth

BY

G. E. BLACKMAN AND J. N. BLACK[1]

(*Department of Agriculture, Oxford*)

With one Figure in the Text

ABSTRACT

Previous investigations in southern England on twenty-two herbaceous species have demonstrated that for widely spaced plants the diurnal solar radiation limits the net assimilation rate of all species and restricts the relative growth rate of many. In examining how far these limitations apply to other environments it is now shown that in the subtropics and tropics the levels of net assimilation rate and relative growth rate can greatly exceed those so far recorded for cool temperate regions, and these differences are attributed to the higher insolation and temperatures.

From a variety of evidence it is concluded that as the distance between plants is reduced so the net assimilation rate is progressively diminished even in regions of high insolation through the enhanced mutual shading. In consequence levels of light which may be supra-optimal for relatively isolated individuals may yet limit the dry-matter production of a dense population. There is an optimal ratio of leaf area to ground surface (leaf-area index) for the maximal exploitation of the incoming radiation in carbon fixation by the population and this optimum will vary with the species and the light intensity. Where other environmental factors are favourable, light may limit dry-matter production everywhere.

On an annual basis dry-matter production will be dependent on two components—the length of the 'growing season' and the period over which the leaf-area index remains optimal. In the tropics the highest annual rate of production so far recorded is 78 tonnes/hect. produced by *Saccharum officinarum* and in north-east Europe 23·5 tonnes by *Fagus sylvatica*. Over short periods the rate of dry-matter production can attain 38 g./m.^{2}/day and the utilization of solar energy can be as high as 4·2 per cent., or 9·5 per cent. for the range 4,000–7,000 Å.

Although information on the productivity of natural communities is still exceedingly scanty, an attempt has been made to interpret the general pattern in terms of the length of the growing season, the level of solar radiation, the magnitude of the leaf-area index of the whole community, and the period over which the leaf canopy remains green. It is postulated that in any region the vegetation reaches a dynamic equilibrium when there is the maximum exploitation of the incoming radiation to produce the greatest production of dry matter.

INTRODUCTION

IN previous papers of this series—Blackman and Rutter (1948), Blackman and Wilson (1951 *a* and *b*), Blackman, Black, and Kemp (1955), Blackman and Black (1958)—the effects of shading on the vegetative growth of twenty-two herbaceous species have been examined. For eighteen species, including

[1] Present address: Waite Agricultural Research Institute, University of Adelaide.

[Annals of Botany, N.S. Vol. 23, No. 89, 1959.]

species associated with shady habitats, it was concluded that under the conditions of the southern half of England the net assimilation rate is limited by the level of light received per day, even though the mean intensity can exceed 2,000 foot-candles. For twenty-one out of the twenty-two species it was demonstrated that slight shading (0·5 daylight) always reduced the relative growth rate. On the basis of the response curves it was concluded that the growth rate of many species is restricted even in midsummer by the amount of light energy. Blackman and Wilson (1951 *a* and *b*) in discussing how far the earlier findings were applicable to other regions pointed out that in high summer between the tropics and the sub-arctic the fall in the mean light intensity is to some extent offset by the gain in day length. They also emphasized that in many parts of the world, particularly in the tropics and sub-tropics, there was a great paucity of data on either the growth rate of individual species or the extent to which growth might be determined by the daily radiation. Finally, Black (1957) in his review of the influence of varying light intensity on the growth of herbage plants stressed that a distinction must be drawn between the reactions of plants grown as isolated individuals or in the mass, as in swards.

This paper is an attempt at a general synthesis of the factors which operate in determining the conditions under which light may limit growth or dry-matter production by either individuals or populations of plants.

Interrelationships between Plant Density, Self-Shading, and Growth Responses to Light

In all the experiments of this series of investigations the plants were grown widely spaced apart, so that shading of one individual by another was kept to a minimum. In addition, to avoid within limits the shading of one leaf by another on the same plant the experiments were conducted when the plants were small. The magnitude of the effects of self-shading has not always been appreciated and is clearly a complicating factor in comparisons either between species or workers. As the plant becomes taller or as the density is increased, so self-shading is enhanced, and there may be an exceedingly steep light gradient between the top and bottom of the plant. Brougham (1956) has recorded that when a ryegrass-clover pasture was 13 cm. high then at 3 cm. above the ground the light intensity was negligible, while in a sward of *Trifolium subterraneum* light absorption can be complete (Black, 1958). In America and Europe similar measurements have been made of the proportion of light penetrating cereals crops, and with a combination of a high density and a high level of nutrients the amount reaching the soil surface may be as little as 2–6 per cent.: with some other crops, e.g. *Linum usitatissimum*, much more light is transmitted (see Black, 1957). There appear to be few systematic and critical investigations of the light gradient in natural communities. Most attention has been given to woodland where the mean level of light on the forest floor in temperate and tropical regions is of the same order as that under many crops (e.g. Blackman and Rutter, 1946, Evans, 1939, and Ovington and

Madgwick, 1955). In addition Monsi and Saeki (1953), examining a wide range of Japanese herbaceous communities, found that in the majority the light intensity at ground level in the summer was only 2–3 per cent. of daylight.

Hodgson and Blackman (1957) have analysed in some detail the influence of varying density and stage of development on the light gradient in *Vicia faba*. At high densities (55–65 plants/m.^{2}) the gradient reaches its maximum in the flowering phase, when at ground level the intensity falls as low as 0·03 daylight. At this stage it was estimated that 38 per cent. of each plant was at or below the compensation point. When the density was reduced to 11–12 plants/m.^{2} the light gradient was markedly altered: the minimal intensity even at the base of the plant was above the compensation point.

From the foregoing it is evident that comparisons of either the effects of shading or differences in regional or seasonal radiation on the components of growth will only be valid if the degree of self-shading can be taken into account in the different investigations. This aspect is equally important in the extrapolation of data for isolated leaves or shoots to the photosynthesis of whole plants in the field. With such leaves or shoots self-shading is negligible, and under these conditions it would seem from the findings of a number of workers that species vary widely in their light requirements. At one end of the scale are the so-called shade plants, such as Dryopteris or *Saintpaulia ionantha*, which attain their maximum rate of photosynthesis at or below 1,000 foot-candles. Next come a group, including *Linum usitatissimum*, where the corresponding intensity is between 1,000 and 2,000 foot-candles, and this is followed by a large class, containing many crop plants, in which light saturation is reached at between 2,000 and 3,000 foot-candles. For other species the light requirement is somewhat higher, 3,000–6,000 foot-candles are demanded (Singh and Lal (1935), Kramer and Decker (1944), Verduin and Loomis (1944), Bourdeau (1954), and Böhnung and Burnside (1956)).

From this type of result it has been argued for many species that much of the daily radiation is 'wasted', in the sense that the energy levels are above the saturation threshold for photosynthesis. Bormann (1956) has estimated that in the eastern United States in high summer 44·5 per cent. of the light may have an intensity above 6,000 foot-candles, which is the mean saturation intensity for some forest trees of the region. He therefore concluded that slight shading would depress photosynthesis in the early morning and late afternoon, but not at mid-day. Such a conclusion fails to take into account the undoubted light gradients within the canopy and the fact that while intensities above 6,000 foot-candles would not alter the assimilation of the most exposed leaves they could still enhance the carbon fixation of the shaded leaves. This point has been very clearly demonstrated by Müller (1951), who studied the photosynthesis of *Hordeum vulgare* at several stages of development. To simulate field conditions the pots were sunk flush in the ground and the surrounding area sown at the same time. When the plants were in the vegetative phase and there was little self-shading the light-saturation point was

between 25,000 and 30,000 lux, but 5 weeks later when the leaf area had more than doubled the rate of photosynthesis was still increasing at intensities above 50,000 lux. This change in the level of light saturation Müller ascribed to increased mutual shading. The alternative explanation that the photosynthetic activity markedly alters with age is not in agreement with the results of Gregory (1926), who found that the net assimilation rate in the same species is little affected by the stage of vegetative development. For *Helianthus annuus* Blackman and Wilson (1951*a*) reached a similar conclusion.

Thomas and Hill (1949) have also investigated the photosynthesis of crops in the field where there must have been a high degree of self-shading. They established that the net photosynthesis did not attain a maximum value for *Beta maritima*, *Medicago sativa*, and *Triticum vulgare* until the intensity reached 4,400, 4,700, and 5,300 foot-candles respectively. In contrast Went (1957) records that for seedlings of *B. maritima* under conditions where there was no self-shading light saturation in environmental chambers occurred at 1,000 foot-candles. This divergence would suggest that with a high degree of self-shading the optimal light level may change at least fourfold. In parenthesis, it should be pointed out that the levels of light saturation put forward by Went for a number of species are lower than those advanced by other workers. For example, Böhnung and Burnside (1956) record that the photosynthesis of *Solanum esculentum* does not attain a maximum below *c.* 2,000 foot-candles, while Went gives 1,000 foot-candles as the saturation value.

Watson (1958) has also obtained evidence in the field at Rothamsted that in normal stands of *B. maritima* and *Brassica oleracea acephala* self-shading depresses photosynthesis. When the stands were reduced by 25, 50, and 75 per cent. then during the next 10–14 days the net assimilation rate was inversely related to density.

Langer (1957) has emphasized that in the early vegetative phase of *Phleum pratense* estimates of net assimilation rate derived from changes in the shoot are misleadingly low. Inspection of data from sunflower experiments at Oxford shows that over a considerable range of light level exclusion of the roots gives on average an underestimate of 40–45 per cent. but that the corresponding relative growth rate is little affected since the root weight ratio is largely independent of seasonal variations in diurnal radiation and, within limits, of plant size. With these considerations in mind it is of interest to examine the growth data recorded by Clements, Weaver, and Hanson (1929) for spacing experiments on *H. annuus* carried out in Nebraska, a region of high solar radiation. It should be emphasized that the records for both the dry weight and the leaf area from which the estimates of net assimilation rate have been calculated by us were for the shoot only and that the plots were unreplicated. Nevertheless it is clear from Fig. 1 that for three successive periods increasing the density has caused consistent and major depressions in the net assimilation rate. It does not necessarily follow that these depressions were solely dependent on increasing self-shading with rising density but the authors state that in the initial phase 'drouth did not complicate

conditions'. The data for height, also given in Fig. 1, lend support to the conclusion that increasing competition for light rather than for water was more important, since the most widely spaced plants were never the tallest. Since, too, the relative growth rates of the plants at the lowest densities were high, it is most improbable that the major reductions in assimilation at high densities could be ascribed to increased competition for nutrients, as unless deficiencies in the major elements are great the net assimilation rate is not largely affected.

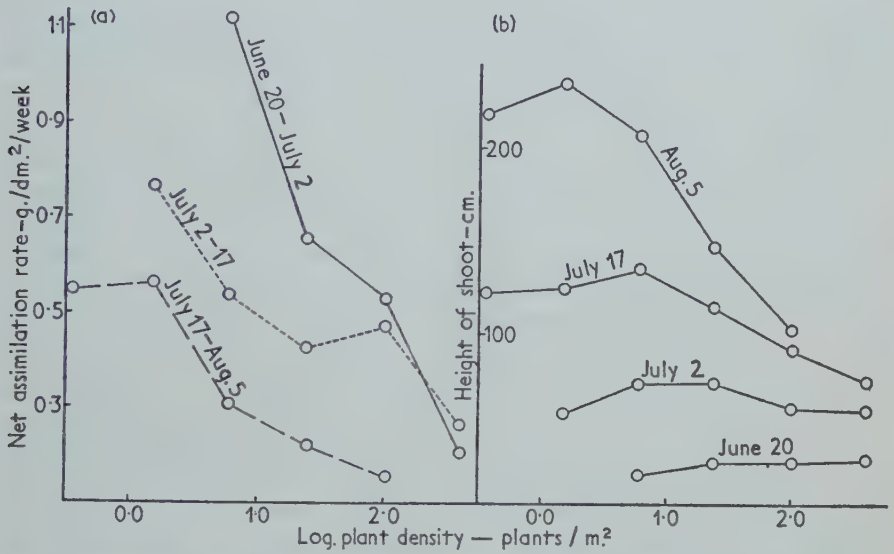


FIG. 1. *Helianthus annuus.* The influence of varying density on (*a*) the net assimilation rate and (*b*) height of individual plants over successive periods of growth.

In Fig. 1 the highest value of the net assimilation rate, despite the underestimation already discussed, is well above the 'constant' mean range of 0·4–0·7 gm./dm.²/week postulated by Heath and Gregory (1938) for all species under either tropical or temperate conditions. These authors recognized that variation in the degree of self-shading was a possible complicating factor and considered that the low value for *Lolium multiflorum* of 0·32 might be due to self-shading. It is therefore pertinent to note that in a previous investigation (Blackman and Black, 1959) with widely spaced plants of the same species a level of 0·82 was recorded.

The Levels of Net Assimilation Rate and Relative Growth Rate under Conditions of High Insolation

Techniques of growth analysis have rarely been applied outside the cool temperate regions, and Gregory and Heath were only able to cite data, and then for the shoots only, of one species (*Gossypium*) for two regions of high insolation (Sudan and South Africa). Evidence is, however, slowly accumulating

that the maximum levels of assimilation and growth rate of whole plants can be very high in such regions, and the information which has been collected is set out in Tables 1 and 2. In this connexion the authors are indebted to Dr. G. L. Hodgson, Dr. E. Njoku, and Dr. G. L. Wilson for allowing them to include unpublished data from the Scottish Horticultural Institution, the University of Nigeria, and the University of Queensland.

Considering first the results for *H. annuus*, it should be pointed out that save for Nebraska the same variety (Pole Star) and the same source of seed from Oxford has been used in each investigation. For the Scottish and English conditions the pairs of experiments cited are those in which either the net assimilation rate or the relative growth rate is the highest so far recorded. The figures for Nebraska have been calculated on a shoot basis from the data of Clements *et al.* (1929).

Inspection of Table 1 shows that at Brisbane and Adelaide the growth rates greatly exceed those found in Great Britain owing primarily to the high leaf-area ratio at Brisbane and the high assimilation rate at Adelaide. At Adelaide, during the course of the experiment, the mean light received per day attained 56,800 foot-candle hours. In this connexion over two seasons Blackman *et al.* (1955) measured in weekly experiments the influence of seasonal changes in light and temperature on the growth of *H. annuus* in England. They found that the net assimilation rate was not significantly affected by temperature, and it is of interest to extrapolate the linear regression on light (upper observed limit of 2,600 foot-candles for a 16·5 hour day) to the high light conditions at Adelaide. The predicted value of 1·19, although an underestimate, is of the right order and would indicate that light saturation, even for widely spaced and young plants, had not been reached, though during the hours of daylight the mean intensity was just over 4,000 foot-candles. In this connexion it is worth commenting that in Böhnung and Burnside's (1956) laboratory studies of leaves or shoots of *H. annuus* enclosed in a chamber a much lower level of light saturation was obtained.

TABLE 1

The Maximal Limits for the Net Assimilation Rate and Relative Growth Rate of Helianthus annuus *in some Environments of High and Low Insolation*

Country	Net assim. rate g./dm.²/week	Leaf-area ratio dm.²/g.	Relative growth rate g./g./day
Oxford, England	0·98	1·10	0·15
Slough, England	0·73	1·70	0·17
Invergowrie, Scotland	0·98	1·32	0·18
,, ,,	1·19	0·95	0·16
Adelaide, Australia	1·36	1·30	0·25
Brisbane, Australia	0·86	1·98	0·26
Nebraska, U.S.A.	1·09	—	0·16

The data for Ibadan (Table 2) again illustrate the magnitude of the growth rate that can be attained under tropical conditions. Indeed, the rate for *Z.*

mays is believed to be the highest ever recorded for a higher plant growing in the open. Moreover, the relative growth rates for the remaining species also exceed the previous known maxima of 0·15–0·205 which have been cited for temperate conditions by Goodall (1945) and Blackman and Wilson (1951*b*). There are good grounds for concluding that, apart from the greater radiation leading to increased assimilation, the higher temperatures positively influence the leaf-area ratio (Blackman, 1956) and hence the relative growth rate.

TABLE 2

The Growth Characteristics of some Species under Environmental Conditions of High Insolation and High Temperature

Country and species	Net assim. rate g./dm.²/week	Leaf-area ratio dm.²/g.	Relative growth rate g./g./day
Ibadan, Nigeria			
Zea mays	1·52	1·69	0·33
Ipomaea caerulea (1956)	0·82	2·37	0·27
,, ,, (1955)	0·69	2·32	0·23
Solanum esculentum	0·91	2·30	0·28
Vigna unguiculata	0·82	1·85	0·21
Lincoln, Nebraska			
Zea mays	0·86	—	0·24
Adelaide, Australia			
Trifolium subterraneum	0·91	1·08	0·13

The net assimilation rate of *Z. mays* is also a record, but the figure is somewhat of an over-estimate since the measured leaf area did not include the green leaf sheaths. Nevertheless, the results for this and the remaining species point to the high level of assimilation. For example, the maximum rate for *S. esculentum* given by Heath and Gregory (1938) was 0·61 for greenhouse conditions in England. In parenthesis, the highest growth rates that have been previously noted for this species have been under glass, for England 0·24 (Goodall, 1945) and 0·285 for Brisbane (Wilson, private communication).

Included in the table are additional data for *Z. mays* which have been calculated from Kiesselbach's (1950) field studies of the seasonal growth in Nebraska. Since only measurements for the shoot were recorded, there is some degree of error, but it is apparent that the growth rate is again very high. Since the plants were singled to clumps of two, the net assimilation rate, apart from the underestimation due to the exclusion of the roots, will have also been reduced by some degree of self-shading. Lastly, the data for *T. subterraneum* (for fuller details see Black, 1955) serve to emphasize the diversity of the characteristics of species. Though statistical analyses showed that light was the principal factor limiting seasonal growth, neither the net assimilation rate nor the growth rate ever approached those of *H. annuus* grown in comparable experiments.

From the foregoing discussion it can only be concluded from the several

different approaches that in cool temperate regions the daily radiation even at the height of the summer is limiting the photosynthesis of many species, even when self-shading is negligible. The new data from areas of high insolation emphasize that the assimilation rates of all the species examined can be outside the normal range experienced in temperate climates. At what level of radiation the light factor ceases to be limiting under conditions where there is little self shading will require further investigation, but the evidence for *H. annuus* and *T. subterraneum* suggests that at Adelaide light saturation has not been reached even in midsummer. Considering that at Adelaide the highest monthly means for solar radiation lie within the range of 600–650 cal./cm.^{2}/day and that in any part of the world the corresponding maximum rarely exceeds 750 cal./cm.^{2}/day (Black, 1956), it follows that if there is a high degree of self-shading then everywhere light will ultimately limit the assimilation of species like *H. annuus*. Again, since Thomas and Hill (1949) found that in close stands of *B. maritima*, *M. sativa*, and *T. vulgare* assimilation was not maximal until the intensity had attained 4,400–5,300 foot-candles, it can reasonably be concluded that in many of the countries where these species are grown light will be the final factor controlling the production of dry matter.

Dry-Matter Production and the Efficiency of the Utilization of Solar Energy

Clearly the optimal utilization of solar radiation will not come about unless there is a maximal absorption by the leaves, and this will be linked with the leaf-area index, that is, the total leaf area per unit of ground. There is evidence from Watson (1958) and Brougham (1958) that the optimal index is dependent on the species, and it is reasonable to conclude that the habit of the shoot and the arrangement and shapes of the leaves will determine the nature of the light gradient. It can also be postulated that, under conditions where growth is not restricted by temperature or by supplies of nutrients of water, maximal production of dry matter attainable per unit area will be limited by the leaf-area index and the amount of solar radiation.

Since outside north-east Europe almost no attempt has apparently been made to measure precisely the maximal rates of dry-matter production of individual species in different environments, estimates have to be derived from data of other types of investigation in many fields. No endeavour has been made to survey exhaustively the very scattered literature but rather to concentrate on a few species which have been widely investigated and thus perforce on species of agronomic importance: even so there is a great paucity of basic information. The results which are cited in Table 3 serve to illustrate the order of the maximal levels of production that can be achieved: it may well be that even higher figures have been missed in the survey. It should, however, be emphasized that data for other species have been examined, but the figures are all below those quoted in Table 3.

TABLE 3

Maximal Rates of Dry-Matter Production per Unit Area of Selected Species in some Tropical and Temperate Regions, together with some Estimates of the Efficiency of the Utilization of Total Solar and Light Energy in Carbon Fixation

Species and country	Rate of dry-matter production g./m.²/day	Per cent. utilization of (*a*) total radiation	Per cent. utilization of (*b*) light energy (4,000–7,000 Å)
**Saccharum officinarum* (Hawaii)[1]	38	—	—
Zea mays (New Hampshire, U.S.A.)[2]	27	—	—
,, ,, (Nebraska, U.S.A.)[3]	24	—	—
Gossypium sp. (Georgia, U.S.A.)[4]	27	—	—
Beta maritima (England)[5]	31	4·2	9·5
Hordeum vulgare (England)[6]	23	1·9	4·3
Brassica oleracea acephala (England)[7]	21	2·2	4·9
	Tonne/hect./year		
Saccharum officinarum (Hawaii)[8]	78	—	—
,, ,, (Hawaii)[9]	72	1·4	3·2
Fagus sylvatica (Denmark)[10]	23·5	1·5	3·5
Pinus sylvestris (England)[11]	22	1·1	2·4
Helianthus tuberosus (England)	20	—	—
Solanum tuberosum (Eire)[12]	18	—	—

* Data cited or calculated from [1]Borden (1942), [2]Hannover (1888), [3]Kiesselbach (1950), [4]Olson and Bledsoe (1942), [5,7]Watson (1958), [6]Watson (private communication), [8]Borden (1945), [9]Burr *et al.* (1957), [10]Möller, Müller, and Nielsen (1954), [11]Ovington (1957), and [12]Keeble (1932).

It would seem that at the optimal point in the growth cycle *S. officinarum* produces more dry matter than any other species for which records have been found. From the data of Ozawa (1933) it can be calculated that under the conditions of Taiwan the leaf-area index can exceed five. The somewhat lower figures of the maximal rate for *Z. mays* and *Gossypium* sp. could be due to a variety of causes. For example, the mean radiation for high summer in Nebraska (Lincoln) and in Florida (Gainesville) is lower than that in Hawaii—see Hand (1937) and Crabb (1950). In the Nebraska experiment the leaf-area index was also low, since the highest value attained was 1·64. It is of considerable interest that the maximal rate for *B. maritima* is second to that of *S. officinarum*. The leaf-area index was again high (five), but on the basis of the diurnal radiation data supplied by Dr. J. L. Monteith of Rothamsted Experimental Station, the mean radiation was only 294 cal./cm.²/day and the assimilation of even the most exposed leaves must have been light-limited.

From the radiation data, and given the energy released on the complete combustion of the dry matter, estimates of the efficiency of the utilization of the incoming solar energy can be made. For *B. maritima*, *H. vulgare*, and *B. oleracea acephala*, which all contained a high percentage of green leaf, a mean figure of 4,000 cal./gm. has been taken and this is probably an underestimate. From Table 3 it is evident that the efficiency of 4·2 per cent. for *B. maritima*

is much in excess of the usually quoted 1–2 per cent. According to Moon (1940) the proportion of the total incoming energy which lies between 4,000 and 7,000 Å at air mass 2 is 44 per cent.[1] and on this basis the efficiency of light utilization is no less than 9·5 per cent. The corresponding figures *H. vulgare* and *B. oleracea* are somewhat lower, but nevertheless in terms of light energy the mean efficiency is 4·6 per cent.

The levels of dry-matter production on an annual basis show *S. officinarum* in the most favourable environments to be again the most productive of species. In fact, the levels cited for Hawaii compare favourably with the maximum yields, estimated by Tamiya (1957), for the mass culture of algae out of doors. It would be anticipated that in cool temperate regions with a season of active growth restricted to 6–7 months the productivity will be lower. Under these conditions the production of stands of the most favoured species of forest trees can, at their peak, produce more than populations of herbaceous species.

Any reasons advanced to explain these differences must be largely speculative. *P. sylvestris*, as an evergreen, can assimilate throughout the year when conditions allow, and Rutter (1957) has shown that young trees can grow, albeit very slowly, during the English winter. On the other hand, between bud break and leaf fall *F. sylvatica* will have received a somewhat higher level of radiation in Denmark. Then again, apart from any specific difference in the net assimilation rate, the shade cast by a beech wood, once the canopy is closed, is very deep and the leaves are retained until late in the autumn. This extended period when the leaves are green and the leaf-area index is high is almost certainly one of the main reasons for high productivity of *H. tuberosus*, recorded in experiments at Oxford. Similarly, a cool humid environment with a long period free from frost, such as may be found in Eire, will provide optimal conditions for *S. tuberosum*.

At this juncture it is of interest to consider the investigations of Pearsall and Gorham (1956), and Pearsall and Newbould (1957). They have sought to assess the seasonal production of dry matter in different communities in Great Britain and Lapland. The method adopted has been to collect in the late summer from sample quadrats the new shoots which have been formed since the spring and after drying to weigh the produce. The authors have themselves drawn attention to the deficiencies of this technique since no account is taken of comparable changes in the roots, rhizomes, or other storage organs of the perennial species. There is another difficulty that needs emphasis, namely the timing of the sampling. For many perennial species it has been demonstrated that much of the early shoot growth is at the expense of

[1] The ratio will be affected by the amount of cloud and the levels of dust and water vapour in the atmosphere, but Moon suggests that his values for an air mass of 2 are the most appropriate for general use. In this connexion comparisons between a recorder integrating the light between 4,000 and 7,000 Å (for details see Blackman, Black, and Martin, 1953) and a Kipp solarimeter do not suggest that the variation due to cloud is large, since the ratio of the diurnal totals of the two instruments is not very different on bright and cloudy days. A similar conclusion is reached by Trickett, Mowlsley, and Edwards (1957).

root or shoot reserves and that the times of the maximal shoot and plant weights do not coincide, e.g. *Endymion non-scriptus*. By and large there is little doubt that this estimate of shoot growth underestimates the seasonal fixation of carbon.

In Great Britain the seasonal production ranged from 4 to 10 tonnes per hectare. Communities dominated, for example, by *Molinia caerulea* or *Carex arenaria* were the least productive, while at the other end of the scale the dominants were, for example, *Pteridium aquilinum* or *Typha latifolia*. On the basis of the concepts under discussion these differences can be attributed to variations in the leaf-area index and the period over which the green canopy persists.

Under the most favourable conditions Pearsall and Gorham (1956) found that shoot production by *P. aquilinum* could reach 14 tonnes per hectare and they suggested that the gain in rhizome weight might be as high as 7 tonnes per hectare. Thus, *P. aquilinum* would equal the production of *H. tuberosus*, and this is not surprising when it is considered that the leaf canopy of *P. aquilinum* can be very dense and is maintained from June to the time of the autumn frosts.

In Lapland the shoot productivity of the communities examined by Pearsall and Newbould (1957) averaged 2·5 tonnes per hectare with a maximum of 4 tonnes and in comparing these figures with others for northern Britain the authors emphasized that, though the mean temperatures during the growing season were not dissimilar, the length of the season was halved in Lapland and the total hours of daylight reduced by a third. On the other hand there is evidence that between May and August the mean solar radiation is of the same order (see Black, 1956). Thus the productivity data for the two regions are consistent with the view that the level is primarily determined by the diurnal radiation and the course and density of leaf production.

Included in Table 3 is the figure arrived at by Burr *et al.* (1957) for the mean utilization of solar energy by *S. officinarum* over the year, and to this has been added a further estimate of light utilization based on the ratio of light to total solar energy. Since the optimal rate of dry-matter production by *P. sylvestris* is maintained over a number of years, some not-too-inaccurate measure of light utilization can be made, given the mean annual solar radiation and the calorific value of the dry matter. The most complete records for solar radiation on a horizontal surface in England are those from Kew Observatory, and since the more recent data from Cambridge are of the same order, the annual mean between 1947 and 1957 of 263 cal./cm.^{2}/day (kindly supplied by Dr. J. M. Stagg) for Kew has been taken as applicable to the plantations in Norfolk examined by Ovington (1957). On this basis, and taking the calorific value as 4,500 cal./g., it is seen from Table 4 that the efficiencies are somewhat lower than those for *S. officinarum*.

Comparable estimates for *F. sylvatica* have been derived by again assuming a calorific value of 4,500 cal./g., and that the leaves are assimilating for six months (May–October). The monthly means of radiation are those of Aslyng

and Kristensen (1958) for a period of five years in the region of Copenhagen. On this basis, the efficiency of *F. sylvatica* is higher than that of *S. officinarum*.

Admittedly, many more comparisons are demanded, but during favourable periods for growth it could be advanced that the efficiency of light utilization should be less in regions of high insolation. Under such conditions the light received by the uppermost leaves may be approaching, or at, the saturation level for photosynthesis.

Finally, the evidence that has been presented leads to the general conclusion that when nutrients and water are in adequate supply then in any community characterized by a high leaf-area index the rate of growth of the vegetational mass will be dependent on the amount of diurnal radiation. It would seem that in periods or in regions of high insolation little light will reach the soil surface if the leaf-area index exceeds five. The form of the light gradient will be dependent on the structure of the vegetation. In some types of community the tops of the shoots of the majority or of all of the species will be subjected to full daylight, while the bases may receive a level of light which is below the compensation point. In other communities where the species occupy different height classes the canopies at the several levels are generally open; in fact for this structure to develop it would seem a pre-requisite that at least for part of the year a considerable proportion of the incoming radiation must penetrate through the foliage of the tallest species.

It has already been noted that the optimal leaf-area index for dry-matter production is dependent on the species and on *a priori* grounds it would seem that this optimum will be attained when the most shaded leaves are receiving light just above the compensation point. It also follows that for a community of a given structural pattern the optimal index will be dependent on the degree of insolation; that is, the higher the radiation the higher the optimum. Conversely, under conditions of low light or shade, such as is experienced by the ground flora of woodland, the optimum will be shifted to a lower level. Blackman and Wilson (1951*b*) have emphasized that the ability to grow at low light intensities will be directly linked with the magnitude of the leaf-area ratio, while Went (1957) has pointed out that in numerous 'shade' species the pattern of the leaves is so arranged that there is the minimum of self-shading. In other words there will be a maximal exploitation of the available light when the leaf-area index is probably between two and three and the assimilatory tissue is a high proportion of the total plant weight.

Penman (1956) has emphasized that the transpiration of herbaceous species will be maximal when the leaf canopy is closed and, provided that water is freely available to the roots, transpiration is directly linked with the incoming solar radiation. Thus, dry-matter production will be maximal in areas of high insolation as long as the rainfall is such that the plants do not suffer from a water deficit and the temperatures allow of active growth throughout the year. These factors will be found in the tropics under two sets of conditions. Where the rainfall is evenly distributed and lies probably within the range of 1,800–

2,400 mm. per annum, the climax is often evergreen rain forest. Where there are swamps, the productivity should be equally high.

Once the rainfall becomes periodic then, as the dry periods are extended and there is a change first to deciduous forest and then to savannah, the leaf-area index will be maintained at high values for successively shorter intervals. Thus, under these conditions, save for the flushes of growth, water rather than light will be the limiting factor.

Beyond a certain degree of aridity where the vegetation becomes sparse the leaf cover will at no time reach a level when there is the most effective utilization of the radiation. On the other hand, since water loss from the soil surface is less than that from a canopy of leaves, carbon accumulation by such a xerophytic community will go on for longer, because less of the available water in the soil will be transpired daily. Such considerations may not apply to desert ephemerals where dense stands can sometimes be found, but here optimal seed production may be best achieved by a very high rate of dry-matter production per unit area over a very short time.

The same postulates can be applied to temperate regions where, besides water shortage, conditions of winter will also be restrictive factors. Here again it would seem that the climax vegetation is compounded of these species which collectively maintain a high leaf-area index for the longest periods. For example, if the winters are long or if a hot and dry period in the summer is combined with a mild and humid winter, evergreen species of tree are dominant. As the water availability decreases woodland gives place to grassland, where a high leaf-area index is more seasonal. Again, reservations must be made for areas of very low rainfall where the leaf cover is incomplete. Similarly, in arctic conditions low temperatures are seemingly more limiting than light (Wilson, 1957).

Lastly, it is tempting to conclude that save for extremes of environment the vegetation in any region reaches a dynamic equilibrium when there is the maximum exploitation of the incoming radiation to produce the greatest production of dry matter.

Literature Cited

Aslyng, H. C., and Kristensen, K. J., 1958: Investigations on the Water Balance in Danish Agriculture. II. 1953–7. Royal Vet. and Agric. Coll. Copenhagen, Yearbook, 1958: 64.

Black, J. N., 1955: The Interaction of Light and Temperature in Determining the Growth Rate of Subterranean Clover (*Trifolium subterraneum* L.). Aust. J. Biol. Sci., **8**, 330.

—— 1956: The Distribution of Solar Radiation over the Earth's Surface. Arch. Met. Geoph. Bioklim. (B), **7**, 165.

—— 1957: The Influence of Varying Light Intensity on the Growth of Herbage Plants. Herbage Abstr., **37**, 89.

—— 1958: Competition between Plants of Different Initial Seed Sizes in Swards of Subterranean Clover (*Trifolium subterraneum* L.) with Particular Reference to Leaf Area and the Light Microclimate. Aust. J. Agric. Res., **9**, 299.

Blackman, G. E., 1956: Influence of Light and Temperature on Leaf Growth in 'The Growth of Leaves', ed. F. L. Milthorpe. Butterworth, London.

Blackman, G. E., and Black, J. N. 1959. Physiological and Ecological Studies in the Analysis of Plant Environment. XI. A Further Assessment of the Influence of Shading on the Growth of Different Species in the Vegetative Phase. Ann. Bot., n.s., **23**, 51.

—— Black, J. N., and Kemp, A. W., 1955: Physiological and Ecological Studies in the Analysis of Plant Environment. X. An Analysis of the Effects of Seasonal Variation in Daylight and Temperature on the Growth of *Helianthus annuus* in the Vegetative Phase. Ibid., n.s., **19**, 527.

—— and Rutter, A. J., 1946: Physiological and Ecological Studies in the Analysis of Plant Environment. I. The Light Factor and the Distribution of the Bluebell (*Scilla non-scripta*) in Woodland Communities. Ibid., **10**, 361.

—— —— 1948: Physiological and Ecological Studies in the Analysis of Plant Environment. III. The Interaction between Light Intensity and Mineral Nutrient Supply in Leaf Development and in the Net Assimilation Rate of the Bluebell (*Scilla non-scripta*). Ibid., **12**, 1.

—— and Wilson, G. L., 1951*a*: Physiological and Ecological Studies in the Analysis of Plant Environment. VI. The Constancy for Different Species of a Logarithmic Relationship between Net Assimilation Rate and Light Intensity and its Ecological Significance. Ibid., **15**, 63.

—— —— 1951*b*: Physiological and Ecological Studies in the Analysis of Plant Environment. VII. An Analysis of the Differential Effects of Light Intensity on the Net Assimilation Rate, Leaf-area Ratio, and Relative Growth Rate of Different Species. Ibid., **15**, 373.

Böhnung, R. H., and Burnside, C. A., 1956: The Effect of Light Intensity on Rate of Apparent Photosynthesis in Leaves of Sun and Shade Plants. Amer. J. Bot., **43**, 557.

Borden, R. J., 1942: A Search for Guidance in the Nitrogen Fertilization of the Sugar Cane Crop. 1. The Plant Crop. Haw. Plant Rec., **46**, 191.

—— 1945: The Effect of Nitrogen Fertilization upon the Yield of and Composition of Sugar Cane. Ibid., **49**, 259.

Bormann, R. H., 1956: Percentage Light Readings, their Intensity-Duration Aspects and their Significance in Estimating Photosynthesis. Ecol., **37**, 473.

Bourdeau, P., 1954: Oak Seedling Ecology Determining Segregation of Species in Piedmont Oak-Hickory Forests. Ecol. Monographs, **24**, 297.

Brougham, R. W., 1956: Effect of Intensity of Defoliation on Regrowth of Pasture. Austr. J. Agric. Res., **7**, 377.

—— 1958: Interception of Light by the Foliage of Pure and Mixed Stands of Pasture Plants. Ibid., **9**, 39.

Burr, J. O., Hart, C. E., Brodie, H. W., Tanemoto, T., Kortschak, H. P., Takahaski, D., Aston, F. M., and Coleman, R. E., 1957: The Sugar Cane. Annu. Rev. Plant Physiol., **8**, 275.

Clements, F. E., Weaver, J. E., and Hanson, H. C., 1929: Plant Competition. Carnegie Institution of Washington, 1929.

Crabb, G. A., 1950: Solar Radiation Investigations in Michigan. Michigan Agric. Exp. Sta. Tech. Bull. **222**.

Evans, C. C., 1939: Ecological Studies on the Rain Forest of South Nigeria. 2. Atmospheric Environmental Conditions. J. Ecol., **27**, 436.

Goodall, D. W., 1945: The Distribution of Weight Change in the Young Tomato Plant. Ann. Bot., n.s., **9**, 101.

Gregory, F. G., 1926: The Effect of Climatic Conditions on the Growth of Barley. Ibid., **40**, 1.

Hand, I. V., 1937: Review of United States Weather Bureau Solar Radiation Investigations. Month. Weath. Rev., **65**, 415.

Hannover, N. H., 1888: When to Cut Corn for Silage. New Hampshire Agric. Exp. Sta. Bull. **3**.

Heath, O. V. S., and Gregory, F. G., 1938: The Constancy of the Mean Net Assimilation Rate and its Ecological Importance. Ann. Bot., n.s., **2**, 811.

Hodgson, G. L., and Blackman, G. E., 1957: An Analysis of the Influence of Plant Density on the Growth of *Vicia faba*. 2. The Significance of Competition for Light in Relation to Plant Development at Different Densities. J. Exp. Bot., **8**, 195.

Keeble, F., 1932: Fertilizers and Food Production on Arable and Grassland. Oxford University Press, London.

Kiesselbach, T. A., 1950: Progressive Development and Seasonal Variations of the Corn Crop. Nebraska Agric. Exp. Sta. Res. Bull. **166**.

KRAMER, P. J., and DECKER, J. P., 1944: Relation between Light Intensity and Rate of Photosynthesis in Loblolly Pine and Certain Hardwoods. Plant Physiol., **19**, 350.
LANGER, R. H. M., 1957: Growth and Nutrition of Timothy (*Phleum pratense*). 2. Growth of the Plant in Relation to Tiller Development. Ann. Appl. Biol., **45**, 528.
MÖLLER, C. M., MÜLLER, D., and NIELSEN, J., 1954: Graphic Presentation of Dry-Matter Production of European Beech. Forstl. Forsøksv. Danm., **21**, 327.
MONSI, M., and SAEKI, T., 1953: Über den Lichtfaktor in den Pflanzengesellschaften und seine Bedeutung für die Stoffproduktion. Jap. J. Bot., **14**, 22.
MOON, P., 1940. Proposed Solar Radiation Curves for Engineering Use. J. Franklin Inst., **230**, 583.
MÜLLER, VON D., 1951: Analyse der Stoffproduktion von Gerste. Bodenkultur, **5**, 129.
OLSON, L. C., and BLEDSOE, B. P., 1942: The Chemical Composition of the Cotton Plant and the Uptake of Nutrients at Different Stages of Growth. Georgia Exp. Sta. Bull. **222**.
OVINGTON, J. D., 1957: Dry-Matter Production by *Pinus sylvestris* L. Ann. Bot., N.S., **21**, 287.
—— and MADGWICK, H. A. I., 1955: A Comparison of Light in Different Woodlands. Forestry, **28**, 141.
OZAWA, S., 1933: Biochemical Studies on Maturity of Sugar Cane. 5. The Utilization of Solar Energy. Mem. Coll. Sci. (A), Kyoto Imperial University, **16**, 78.
PEARSALL, W. H., and GORHAM, E., 1956: Production Ecology. I. Standing Crops of Natural Vegetation. Oikos, **7**, 193.
—— and NEWBOULD, P. J., 1957: Production Ecology. IV. Standing Crops of Natural Vegetation in the Sub-Arctic. J. Ecol., **45**, 593.
PENMAN, H. L., 1956: Weather and Water in the Growth of Grass in 'The Growth of Leaves', ed. F. L. Milthorpe. Butterworth, London.
RUTTER, A. J., 1957: Studies in the Growth of Young Plants of *Pinus sylvestris* L. I. The Annual Cycle of Assimilation and Growth. Ann. Bot., N.S., **21**, 399.
SINGH, R. N., and LAL, K. N., 1935: Limitation of Blackman's Law of Limiting Factors and Harder's Concept of Relative Minimum as Applied to Photosynthesis. Plant Physiol., **10**, 245.
TAMIYA, H., 1957: Mass Culture of Algae. Annu. Rev. Plant Physiol., **8**, 309.
THOMAS, M. D., and HILL, G. R., 1949: Photosynthesis under Field Conditions in 'Photosynthesis in Plants', ed. Frand and Loomis, Iowa. Iowa State College Press.
TRICKETT, E. S., MOULSLEY, L. J., and EDWARDS, R. I., 1957: Measurement of Solar and Artificial Radiation with Particular Reference to Agriculture and Horticulture. J. Agric. Engng. Res., **2**, 85.
VERDUIN, J., and LOOMIS, W. E., 1944: Absorption of Carbon Dioxide by Maize. Plant Physiol., **19**, 278.
WATSON, D. J., 1958: The Dependence of Net Assimilation Rate on Leaf-Area Index. Ann. Bot., N.S., **22**, 37.
WENT, F. W., 1957: The Experimental Control of Plant Growth. Chronica Botanica Co., Waltham, Mass.
WILSON, J. W., 1957: Arctic Plant Growth. Advanc. Sci., **53**, 383.

Cytological Investigations on *Porphyra umbilicalis* (L.) Kütz. var. *laciniata* (Lightf.) J.Ag.

BY

V. KRISHNAMURTHY[1]

(*Department of Cryptogamic Botany, The University, Manchester* 13)

With two Plates and one hundred and five Figures in the Text

Abstract

1. Vegetative mitosis of *Porphyra umbilicalis* var. *laciniata* is normal, the chromosome number as seen in late prophase being five. Special features of this mitosis are the appearance of several stained chromatin segments in early prophase and the formation of a crescent-shaped group of chromosomes during metaphase and anaphase.

2. Genuine monospores have not been seen in the material investigated.

3. Both spores and spermatia arise by repeated division of a mother-cell and in identical ways. Evidence is presented to show that the first division in the mother-cell forming spores is longitudinal. There is no evidence of reduction division in this division, and the chromosome number in the mother-cell is five.

4. The first division in the germinating spore is mitotic and shows five chromosomes.

5. The *Conchocelis*-phase is haploid throughout and produces 'fertile cell rows' and 'plantlets', but spore liberation has not been observed. It is suggested that the 'plantlets' may grow out directly into the leafy phase or may give rise to spores according to prevailing environmental conditions.

6. No evidence of sexual reproduction has been obtained in the present work and the role of the spermatium in the life-cycle is not clear. It is suggested that further work may profitably be concerned with investigating the nature of the spermatium.

Introduction

IT has been pointed out by Drew (1955) that one of the characteristic features of the algae is the diversity of their life-histories. This, together with some flexibility in the sequence of events in the life-history, has hampered the correct interpretation of the latter. There has also persisted through the years a tendency to interpret algal life-histories in terms of the Hofmeisterian life-cycle of the Pteridophyta, with emphasis on the place of occurrence of reduction division and the association of morphological phases with changes in the nuclear phases. However, fuller and more penetrating investigations have shown that new concepts are necessary for the adequate interpretation of algal life-histories. Thus it is becoming increasingly clear that particular morphological phases in the life-history do not always coincide with particular nuclear phases and that these are often independent of each other (cf. Chadefaud, 1952; Feldmann, 1952).

An account of the life-history of an alga, therefore, should comprise two sections: firstly, information regarding the sequence of morphological or

[1] Present address: Department of Botany, Presidency College, Madras, India.

[Annals of Botany, N.S. Vol. 23, No. 89, 1959.]

somatic phases which can be recognized, and secondly, related information on reduction and syngamy. Drew (1954*a*) has pointed out that this means (1) that the nature of the thallus which develops from the zygote as well as the thalli which develop from all types of asexual spores has to be determined in each instance; (2) the chromosome numbers and the nature of the reproductive organs borne by these thalli have to be ascertained and thereby the number of nuclear phases in the life-history established.

Very few algae have been subjected to such a detailed study and it was with such a complete investigation in mind that Drew (1954*a*) selected for study *Porphyra umbilicalis* (L.) Kütz. var. *laciniata* (Lightf.) J.Ag. As a result of her investigations as well as those of Kurogi (1953*a*, 1953*b*), Arasaki (1954), Suto (1954), Suto and Fujiyama (1952), Suto, Maruyama, and Umebayashi (1954), Takeuchi, Matsubara, Shitanaka, and Suto (1954), Yamasaki (1954), Tseng and Chang (1954–5, 1955, 1956), and Graves (1955), we have now a fairly well-established sequence of somatic phases in the life-history of *Porphyra*, although certain points are still not clear. More information, however, is still needed regarding (1) gametic fusion, the evidence for which is scanty and conflicting, and (2) the nuclear phases. The present paper deals with the results of cytological investigations on *Porphyra umbilicalis* var. *laciniata* with a view to giving a correct interpretation of the life-history of this form. The material used in this investigation is the species used by Drew in her studies, and the name adopted by her in her paper (1954*a*) is adopted here also.

Earlier Investigations

As Drew (1954*a*, 1956) has summarized the literature concerning the life-history of *Porphyra*, only the bare outline of the different somatic phases is given below.

1. The leafy phase of *Porphyra* produces, (1) monospores, (2) 'spermatia', and (3) 'carpospores'. The last are supposed to be formed by repeated division of a cell which is regarded as a fertilized 'carpogonium'.

2. The monospore germinates in a bipolar manner (Dangeard, 1931; Kylin, 1946; Tseng and Chang, 1954–5) and gives rise to the leafy thallus once again.

3. The 'carpospore' on germination gives rise to filamentous growths which on coming into contact with a shell, readily penetrate it and develop into the *Conchocelis*-phase (Drew, 1949, 1954*a*, 1954*b*; Kurogi, 1953*a*; Yamasaki, 1954; Graves, 1955; Tseng and Chang, 1954–5).

4. The *Conchocelis*-phase produces 'fertile cell rows' (Rosenvinge, 1909; Drew, 1954*a*), and the cells give rise to spores (Rosenvinge, 1909; Kurogi, 1953*a*, 1953*b*; Drew, 1954*a*; Arasaki, 1954; Suto, 1954; Suto *et al.*, 1954; Takeuchi *et al.*, 1954; Yamasaki, 1954*b*; Tseng and Chang, 1954–5, 1956; Graves, 1955). Tseng and Chang (1954–5) call these spores 'conchospores', and record the germination of these into leafy thalli. Drew (1954*a*) did not observe either the liberation of these spores or their germination, but recorded the development of what she calls 'plantlets' from the *Conchocelis*-phase.

Dangeard (1931, 1954*a*, 1954*b*) suggested that the *Conchocelis*-phase is protonemal in nature and that it gives rise to the leafy plant as 'pluricellular buds'. Thus there are different views expressed regarding the re-establishment of the leafy phase of *Porphyra*.

Cytological studies on *Porphyra* have been few and the evidence recorded is both scanty and conflicting. Apart from observations of a general nature (Berthold, 1882; Dangeard, 1927), accounts of vegetative mitosis have been given by Ishikawa (1921) and Dangeard (1927). The former considered the process to be intermediate between mitosis and amitosis, while the latter considered it to be normal.

Other workers have sought to present cytological evidence in support of the process of 'fertilization' of a 'female cell' (Joffé, 1896; Dangeard, 1927; Magne, 1952; Tseng and Chang, 1954–5).

Some authors have interpreted the first division leading to the formation of 'carpospores' as constituting a reduction division. Ishikawa (1921) thought that it was a special kind of meiosis by 'fragmentation'. Tseng and Chang (1954–5) have figured what they call 'synapsis'. On the other hand, Magne (1952) thinks that there is no reduction following fertilization and that the carpospores are diploid.

No particulars are available regarding the cytology of the *Conchocelis*-phase.

Material and Methods

This investigation was carried out mainly on *Porphyra umbilicalis* var. *laciniata* collected by the writer from Rhosneigr in Anglesey at various times during 1956–7. This species was also collected at various places in south-west Pembrokeshire in September 1956 and in and around Port Erin in the Isle of Man in April 1957. Besides these collections, the late Dr. K. M. Drew-Baker very kindly placed at the writer's disposal a part of her valuable collections.

Living material of *P. umbilicalis* var. *laciniata* from Rhosneigr was brought to the laboratory and used for starting cultures of the *Conchocelis*-phase. Material was also fixed both in the field and in the laboratory for cytological study. For fixation, a mixture of one part of glacial acetic acid and three parts of absolute alcohol proved to be most satisfactory, although good results were obtained with formalin-alcohol (after Drew, 1934) and formalin-acetic-alcohol. Staining was carried out on whole pieces of the thallus as well as on sections cut at a thickness of 4–6 μ after embedding in paraffin. Squashes were made with aceto-carmine for study of division figures. Other stains used for cytological preparations included iron-alum haematoxylin, brazilin, and crystal violet. Feulgen nucleal reaction was carried out to confirm the results obtained with other staining processes. The reduced basic fuchsin for this reaction was prepared according to a method recommended by Coleman (1938). Hydrolysis was carried out in 12 per cent. hydrochloric acid at 58–60° C. Best results were obtained with material fixed in acetic-alcohol. Hydrolysis time for 'spermatia' was 6–8 minutes while that for 'spores' was

10–15 minutes. Staining was brilliant in 'spermatia' and feeble in other cells. There was a slight staining of the cell walls.

For culture of the *Conchocelis*-phase, the following culture solution suggested by the late Dr. Drew-Baker was used:

Filtered sea water	666 ml.
Unheated soil extract in sea water	333 ml.
Mineral mixture	25 ml.

The mineral mixture was made up as follows:

EDTA (Disodium salt)	0·02 g.
Sodium nitrate	0·20 g.
Sodium hydrogen phosphate	0·04 g.
0·2 per cent. solution of boric acid	1·00 ml.
0·1 per cent. solution of manganese sulphate	0·75 ml.
0·1 per cent. solution of ferric citrate	1·25 ml.
Distilled water	50·00 ml.

The spores to be germinated in culture were obtained from selected spore-producing thalli. Strips from the spore producing margin of the thallus were removed with sterile forceps, rinsed in sterile sea water and then floated in small quantities of culture solution on slides placed in a tray covered with a lid of vita glass. The tray was then placed in a well-illuminated place. The culture solution was replenished twice daily. Spores were liberated in a day or two and showed signs of germination in 4 or 5 days.

The germlings on the slides were fixed from time to time for cytological observations. Some of the slides were also used to 'infect' pieces of sterile shells.

On the shells, the germlings soon penetrated the shell matrix. At this stage, the shells were transferred to beakers of a litre capacity containing 750 ml. of culture solution. The beakers were placed in a culture tank and the cultures were aerated. Illumination was given by five fluorescent tubes suspended over the tank at such a height as to give an intensity of about 3,000 lux. The culture solution was replaced once in two weeks until a rich growth of the *Conchocelis*-phase was obtained and 'fertile cell rows' were formed on it.

The shells containing the growth of *Conchocelis*-phase were fixed in the same way as the leafy thallus. For staining or embedding, the material was decalcified and the softened matrix of the shell and the *Conchocelis*-phase were treated as a whole piece of the thallus.

All preparations were mounted on selected slides of about 0·8 mm. thickness and No. O coverslips were used. Observations were made with a Zeiss apochromatic oil immersion lens of N.A. 1·4, used together with a Holos oil condensor. Kohler illumination was used throughout.

Terminology

The nature and function of the different reproductive bodies in Porphyra are not at all clear. It has been generally assumed that these include (1) mono-

spores, (2) 'spermatia', and (3) 'carpospores'. The nature of the two latter types of reproductive bodies has been assumed to be similar to those of the Florideophycidae ever since Berthold (1882) so interpreted them. However, the evidence for assuming the existence of a sexual mode of reproduction is scanty (cf. Hus, 1902; Rosenvinge, 1909; Drew, 1954*a*, 1954*b*; Dangeard, 1954*b*). Until there is definite evidence that there is a mode of sexual reproduction similar to the one in the Florideophycidae, the nature of these two types of reproductive bodies should be left open. Hence, the writer follows the terminology used by Drew (1954*a*) and refers to these as 'spermatia' and 'spores'. The spores produced by the *Conchocelis*-phase it is proposed to call 'conchospores' after Tseng and Chang (1954–5).

Cytology of the Vegetative Thallus

Gross structure of the thallus

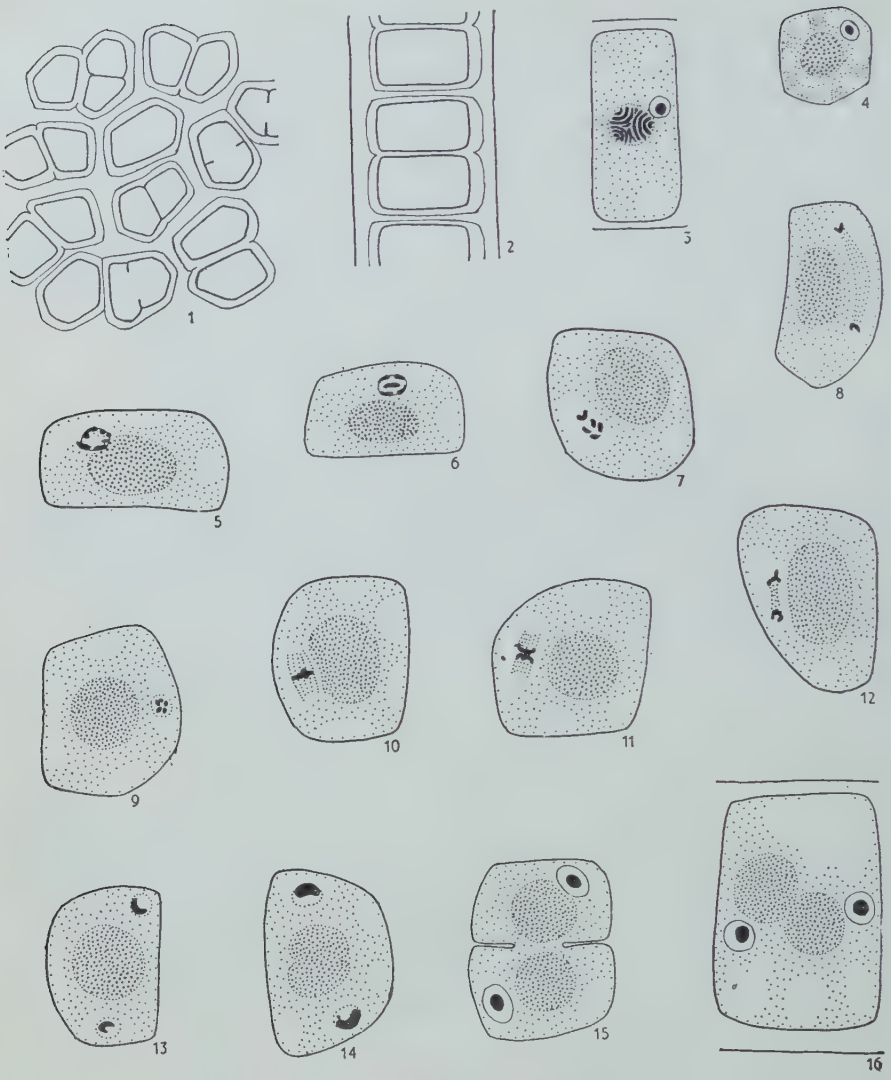
The leafy thallus of *Porphyra umbilicalis* var. *laciniata* grows attached to rocks and stones in the mid-tide level as large olive-green to purple expanses. The attachment is by a small, sessile basal disc of loosely interwoven rhizoids. The basal portion of the thallus is cordate to suborbicular and its margin is divided in varying degrees and is often laciniate. In size it may reach a length of about 50 cm. and a breadth of about 20 cm.

The thallus is monostromatic and consists of cells which are polygonal in outline in surface view (Text-fig. 1). In section, they are rectangular and show a regular parallel arrangement (Text-fig. 2). The cells at the base of the thallus elongate downwards into long, slender rhizoids.

Growth of the thallus is superficial and almost any cell of the thallus may divide by a wall perpendicular to the surface. In young thalli, the marginal cells seem to divide more frequently, but soon divisions become general throughout the surface of the thallus. In surface view, recently divided cells are separated by a very thin septum and so appear as though they are arranged in pairs (Text-figs. 1, 2). Otherwise there appears to be no regularity in the arrangement of cells. The cells vary in size with the age of the thallus. In young plants they measure 6μ–$16\mu \times 6\mu$–$9{\cdot}5\mu$, while in mature plants they are 10μ–$19{\cdot}5\mu \times 7\mu$–$13{\cdot}5\mu$.

Structure of the vegetative cell

The cell wall may be distinguished into an inner and an outer layer surrounding each cell (Text-figs. 1, 2). The different cells are in turn cemented by an amorphous intercellular substance which is continuous with a cuticle-like investment surrounding the entire thallus (Text-fig. 2). The protoplast fills the cell cavity and contains a massive chromatophore which is more or less stellate and includes a large central pyrenoid, and a nucleus on one side between two arms of the chromatophore (Text-figs. 3, 4). Cells in the adult thallus contain a number of small granules of floridean starch which may at times be so abundant as to mask the chromatophore. The large spherical to oval pyrenoid is the most conspicuous object in the cell, being usually a half



TEXT-FIGS. 1–16. *Porphyra umbilicalis* var. *laciniata*. 1. Surface view of small portion of thallus showing arrangement of cells. 2. Cross-section of a portion of thallus. 3. Detail of a single cell from a cross-section of the thallus showing structure of the pyrenoid. 4. A single cell in detail in surface view of thallus. 5. Early prophase nucleus in vegetative cell showing seven stained segments around the nucleolus which is faintly stained. 6. Mid-prophase with five chromosomes around the nucleolus. 7. Late prophase. 8. Late anaphase with curved interzonal spindle. 9. Prometaphase. 10. Metaphase. 11. Early anaphase. 12. Anaphase showing interzonal spindle and crescent shaped groups of chromosomes. 13. Telophase. 14. Commencement of division of the pyrenoid. 15. Cytokinesis. 16. Cell in cross-section of thallus showing division of the pyrenoid after organization of the daughter nuclei.

Figs. 1, 2: ×450; Figs. 3, 4: ×900; Figs. 5–16: ×1,800.

to two-thirds the width of the latter. When stained with iodine or iron alum haematoxylin, it shows a characteristic lamellate structure (Text-fig. 3).

The nucleus of *Porphyra*, as seen in fixed and stained material, is well defined and consists of a fairly prominent nucleolus surrounded by a nuclear material which appears like a clear space in interphase (Text-figs. 3, 4). Although a definite nuclear membrane cannot be seen with the light microscope, the boundary between cytoplasm and nucleus is quite clear owing to difference in intensity of staining with haematoxylin or brazilin. The interphase nucleus is about 3μ in diameter.

Division of the vegetative cell

Division of the vegetative cell of *Porphyra* takes place at all times of the day, but daylight is not an essential requirement, for divisions have been seen in material fixed in the laboratory at 10 p.m.

In early prophase a number of discrete stained bodies are visible towards the periphery of the nucleus. As many as seven such bodies are seen in favourable material, and these are found surrounding a central nucleolus which is feebly stained and ill defined (Text-fig. 5; Pl. 1, Fig. 1). Subsequently only five elongated chromosomes are seen (Text-Fig. 6; Pl. 1, Fig. 2). The stained bodies seen at an earlier stage are presumably heterochromatic segments of some of the chromosomes, which had taken up stain before the rest of the chromosomes became stainable. The five elongated chromosomes are still seen surrounding the nucleolus in mid-prophase, but the nucleolus is now very faintly stained. In a subsequent stage, the nucleolus completely disappears and only the five chromosomes remain as five elongated bodies. These appear like curved rod-shaped bodies surrounding a central space (Text-fig. 7; Pl. 1, Fig. 3). In late prophase and prometaphase, the chromosomes become further condensed and assume a rounded shape. In prometaphase, the chromosomes come together in such a way that in any one plane of focus only three or four of the chromosomes are seen. Occasionally, however, all the chromosomes lie in one focal plane and they can be clearly seen (Text-fig. 9).

Metaphase in vegetative cells has been seen only in side view when it forms a transversely extended rod-shaped structure (Text-fig. 10; Pl. 1, Fig. 4). The appearance of the chromosome plate in polar view could not be ascertained in vegetative cells. But this has been seen in spore-forming cells, where it is in the form of a crescent. This is presumably the situation in vegetative cells also. Side view of metaphase also shows an anastral spindle in the form of very fine spindle fibres extending to a very short distance on either side of the equatorial plate (Text-figs. 10, 11).

In very early anaphase, the chromatids at the extremities of the plate usually separate earlier than those towards the middle (Text-fig. 11), the two crescent-shaped groups subsequently moving apart. During this phase, a distinct interzonal spindle arises, this being the only part of the spindle that persists in later stages (Text-fig. 12; Pl. 1, Fig. 5). Chromosomal fibres are not seen

at all in these stages. As anaphase advances and the chromosome plates move farther apart, a very characteristic event takes place. The interzonal spindle elongates, grows asymmetrically and assumes a curved outline (Text-fig. 8; Pl. 1, Fig. 6). As the chromosome plates thus move in an arc, the positions occupied by the newly organized nuclei at telophase are in a plane different from that of the parent nucleus. Towards the end of anaphase, the chromosome plates appear condensed, forming solid crescent-like bodies and the individual chromosomes are no longer distinct.

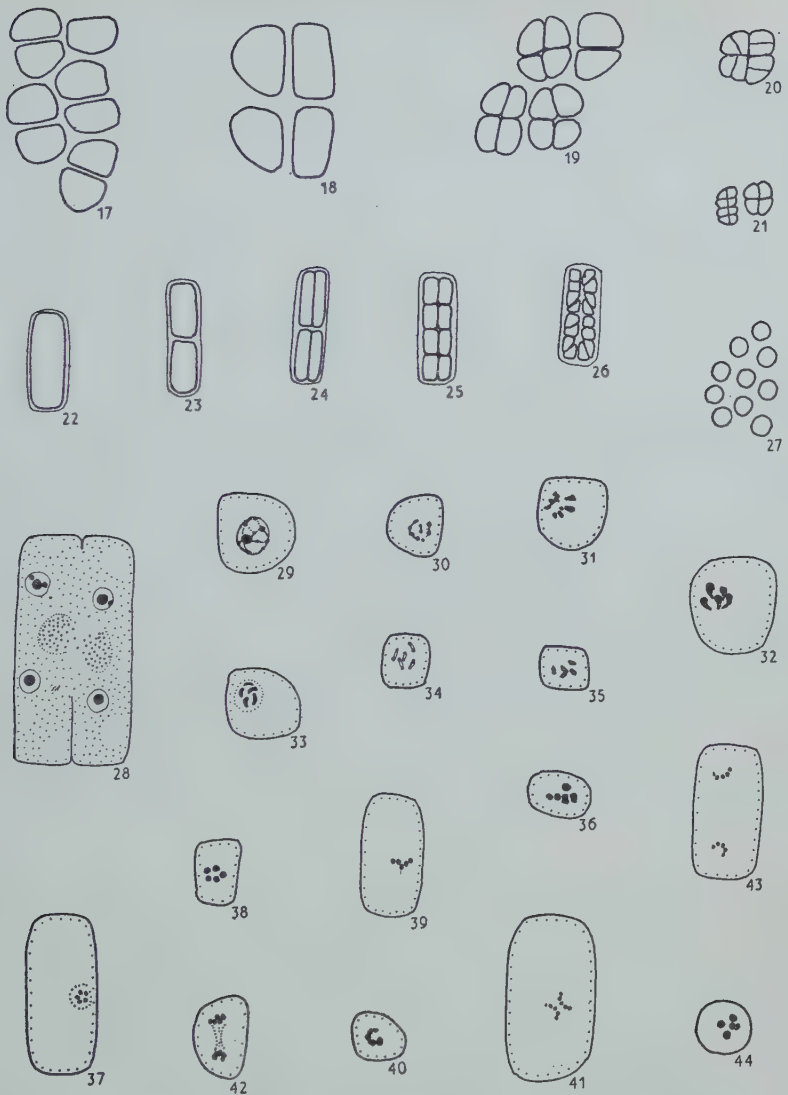
In telophase, the spindle completely disappears. The chromosome plates become condensed and hyaline areas are organized around them (Text-fig. 13; Pl. 1, Fig. 7). The daughter nuclei are soon organized completely.

Cytokinesis then begins, the first part of the protoplast to divide being the pyrenoid (Text-figs. 14, 16). This divides by a constriction in a plane perpendicular to the plane of division of the nucleus. Thereafter, the chromatophore also divides by constriction, this being followed by centripetal cytoplasmic cleavage (Text-fig. 15), and the early formation of a thin pectic wall, extending from the periphery towards the centre. The mucilaginous outer layer also appears in rapid succession. In due course, intercellular substance is laid down between the outer layers of the walls of the daughter cells. The intercellular substance and the outer walls are at first thin, but gradually increase in thickness thereby separating the daughter cells. The nuclei of the daughter cells occupy positions at the farthest sides of the two cells (Text-figs. 15, 16).

Cytology of Formation of Reproductive Cells

Thalli which produce genuine monospores were not found in the collection at the disposal of the writer. However, in thalli producing spores, occasional cells remained undivided, became rounded and were liberated along with the spores. Such cells, however, did not germinate and so their nature could not be ascertained, nor their cytology studied. Therefore, the following account gives the details of cytology of formation of only the spermatia and the spores.

1. *Cytology of formation of spermatia.* Cells of the thallus destined to form the spermatia show an antecedent enlargement (cf. Text-figs. 17 and 18). The enlarged cell then divides by two intersecting walls perpendicular to the surface of the thallus to form a group of four cells arranged in a cruciform manner when seen in surface view (Text-fig. 19). As these walls are parallel to the long axis of the dividing cells, they are hereafter referred to as longitudinal walls. Each of the four cells formed then divides by a wall parallel to the surface of the thallus (Text-figs. 22, 23), hereafter referred to as the transverse wall. In each of the eight cells, two further intersecting longitudinal divisions occur, yielding 32 cells (Text-figs. 20, 21, 24). Each of these cells then divides twice, either transversely or obliquely (Text-figs. 25, 26). A single mother-cell thus undergoes seven successive divisions, 128 spermatia being thereby formed. These then become spherical (Text-fig. 27), and as they ripen the mucilaginous wall layers separating them dissolve and the 128 spermatia



TEXT-FIGS. 17–44. *Porphyra umbilicalis* var. *laciniata*. 17. Cells in surface view of thallus from the vegetative portion. 18. Similar cells from region in which formation of spermatia is commencing, showing enlargement of cells. 19–21. Cells in the spermatium-forming region, showing successive stages of division of the mother-cells. 22–26. Cells in cross-section of thallus from spermatium-forming region showing successive stages in the formation of spermatia. 27. Free spermatia. 28. A spermatium-forming cell showing longitudinal division of the contents and containing nuclei with heterochromatic bodies. 29–43. Cells showing stages of mitosis leading to spermatium formation. 29. Very early prophase with persistent nucleolus. 30. Early prophase where nucleolus has already disappeared. 31. Early prophase showing deeply stained chromatin segments. 32. Prophase showing five chromosomes. 33. Latish prophase. 34, 35. Prophase with five chromosomes showing double strand structure. 36. Late prophase. 37, 38. Prometaphase. 39. Metaphase, side view. 40. Metaphase, polar view. 41–43. Stages in anaphase. 44. A single mature spermatium, showing nucleus in late prophase with five chromosomes.

Figs. 17–27: ×450; Fig. 28: ×900; Figs. 29–44: ×1,800.

remain together in a packet. These are generally liberated by the dissolution of the mucilaginous sheaths surrounding them. The liberated spermatia are 4·5μ–6μ across.

The details of mitosis in the earliest stages of spermatium-formation are difficult to follow for two reasons: (1) the number of cells in which these divisions occur is comparatively small and it is only very occasionally that one is able to obtain cells at this stage; (2) the nuclei of cells at these stages stain poorly and thus render observation of details difficult. Over fifty thalli producing spermatia have been studied and only in one squash preparation was a cell in the early stages of spermatium formation observed. In this cell four nuclei were seen and the commencement of cytokinesis in the longitudinal plane observed (Text-fig. 28). The fact that there are four nuclei in the cell while cytokinesis is observed only in the longitudinal plane suggests that here nuclear divisions occur in advance of cytokinesis, a condition also seen during spore-formation. In the two lateral halves of the cell, there are two nuclei, one towards each surface of the thallus. This can be the case only in cells which have begun the first transverse division and so the cell in consideration must be a half of the spermatium mother-cell.

Of the four nuclei seen in this cell, two are at interphase; each has a single prominent nucleolus with an unstained nuclear material surrounding it and a scarcely visible nuclear membrane. Of the other two nuclei, one shows a single heterochromatic body at the periphery of the nucleus while the other shows two heterochromatic bodies. The appearance of these heterochromatic bodies also resembles the sequence of events in spore-forming cells, to be described later. In fact, there appears to be a close parallel between the modes of formation of spores and spermatia, as far as the earliest stages are concerned.

Later stages in spermatium formation show a number of nuclei in division. Stages of nuclear division have been seen in the third to the seventh cell generation; the process is uniform throughout and ismitotic. Early prophase nuclei are rather feebly stained while the chromosomes of late prophase and the later stages of mitosis stain deeply. This is true also of the intensity of Feulgen nucleal reaction. In the earlier stages of spermatium formation, most cells were observed to have nuclei in early prophase, while in later stages, nuclei in all stages of division were equally abundant.

The nucleus in early prophase shows a well-defined nucleolus and a faint 'reticulum' in which a number of deeply staining bodies are seen (Text-fig. 29). These heterochromatic bodies are often seen as discrete unconnected bodies, but frequently they are interconnected in various ways to form short strings of deeply staining 'granules' (Text-fig. 30; Pl. 1, Fig. 8). In slightly later stages, the interconnexions become more definite and a number of thread-like structures become evident (Text-fig. 31; Pl. 1, Fig. 9). The number of these threads is variable at this stage and hence they cannot be regarded as chromosomes but only as regions of them which become stained earlier and more deeply than the remainder. In later stages, however, five elongated

threadlike chromosomes can be distinguished (Text-figs. 32, 33). The nucleolus, by this time, has completely disappeared. The nucleolus also becomes progressively smaller in successive cell generations and shows a tendency to disappear earlier during prophase of later mitoses. Sometimes the nucleolus is absent even in very early prophase (Text-fig. 30).

In well-differentiated material stained in brazilin, the five chromosomes show characteristic structure. There are two deeply stained bodies at the two extremities of the chromosome, and these are connected by two slender threadlike connexions, giving the aspect of a double strand structure to the chromosomes (Text-figs. 34, 35; Pl. 1, Fig. 10).

In late prophase, the chromosomes condense and become more or less isodiametric (Text-fig. 36; Pl. 1, Fig. 11) and are distributed in more than one focal plane, so that a casual observation does not reveal the number of chromosomes; but careful observation by focusing through the nucleus shows that there are five chromosomes.

In prometaphase, the five chromosomes are arranged in a single focal plane and in this stage it is easy to count them (Text-figs. 37, 38; Pl. 1, Fig. 12).

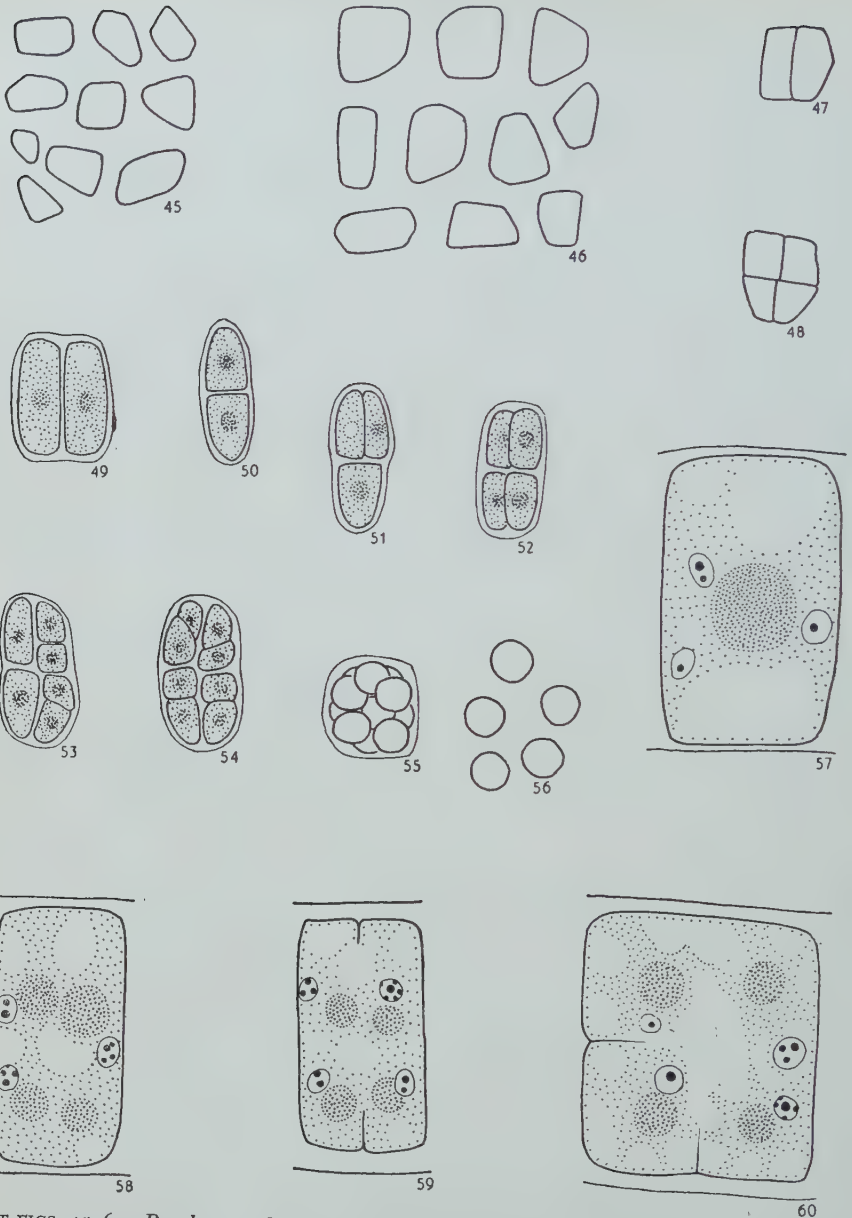
Metaphase, anaphase and telophase follow the pattern seen in vegetative mitosis. These stages are shown in Text-figs. 39 to 43.

The mature spermatia are spherical, include a small pale chromatophore with a pyrenoid and have a nucleus in late prophase with five chromosomes, very much as in some Florideophycidae (Text-fig. 44).

2. *Cytology of formation of spores.* Thalli forming spores can be readily recognized by their rosy margins. This coloration is confined to the spore-forming region of the thallus, the vegetative part being olive-green to purple. If a piece of the thallus is cut across the spore-forming region and examined under the microscope, the sequence of divisions leading to spore formation can be followed. In the region where there is inception of spore formation, there is a general enlargement of the cells (Text-figs. 45, 46). The contents of these cells also show a denser appearance owing to increase in reserve material.

Successive stages of cell division can be observed in successive portions of the thallus towards the periphery. The first division takes place in each of two sister cells formed by the last vegetative division. The first division is by a longitudinal wall (Text-figs. 47, 49). The two daughter cells so formed divide again by a second longitudinal division, in a plane at right angles to that of the first division (Text-fig. 48). As a result four cells are formed which are arranged in a cruciform manner.

It has been pointed out that the daughter cells of a vegetative division are at first separated by a thin wall which later gradually becomes thicker. In the divisions leading to the formation of spores, the cross walls between the products of a single mother-cell remain thin throughout. This feature can be used as a criterion for distinguishing between a vegetative division and a division leading to the formation of spores, as was pointed out earlier by Hus (1902).



TEXT-FIGS. 45–60. *Porphyra umbilicalis* var. *laciniata*. 45. Cells in surface view of vegetative portion of the thallus. 46. Cells from the same thallus, from region of spore formation. 47, 48. Cells in surface view showing successive stages of division leading to spore formation. 49–55. Stages in the formation of spores. 56. Mature spores. 57. Spore mother-cell in cross-section of thallus, showing three of four nuclei (fourth not seen in section) and an undivided pyrenoid. One nucleus shows heterochromatic body. 58. A cell similar to 57, but showing four pyrenoids, but without division of the cytoplasm. All nuclei show variable number of heterochromatic bodies. 59. Spore mother-cell showing first longitudinal cleavage of the cytoplasm. 60. Spore mother-cell showing commencement of a longitudinal as well as a transverse cleavage of cytoplasm.

FIGS. 45–56: ×450; Figs. 57–60: ×1,125.

After the two longitudinal divisions, the protoplast of each of the four cells generally divides by a transverse (Text-fig. 50), or more often by an obliquely transverse wall, but never by a longitudinal wall. Each of the resulting daughter-protoplasts further divides twice in succession (Text-figs. 51–54). Altogether eight segments, each with a thin pectic wall, are formed in each quarter of the original mother-cell. These cells then round up and form the spores. The four groups of spores which are derived from a single mother-cell are at first separated by thin walls. When the spores are mature, these walls disappear and all the thirty-two spores are held together in a single packet (Text-fig. 55). The liberated spore is spherical and is 11μ–$13{\cdot}5\mu$ across (Text-fig. 56).

In sections of this region of the thallus, it can be seen that successive nuclear divisions proceed in advance of cytokinesis, so that, commonly, cells containing two to four nuclei are seen with little or no inception of cytoplasmic cleavage, and often without a division of the pyrenoid (Text-figs. 57, 58; Pl. 1, Fig. 13). The cytoplasm then commences to furrow longitudinally (Text-fig. 59). Sometimes a longitudinal and a transverse furrow commence simultaneously (Text-fig. 60; Pl. 1, Fig. 14). Here, evidently, the nuclear division pertaining to the first transverse division has already taken place, while the cytokinesis of the last longitudinal division is still at its inception. The two divisions, therefore, are evidently both concerned in the process of spore formation and it may be safely concluded that there is at least one longitudinal division prior to the transverse divisions during spore formation. An examination of surface view of spore-forming thalli shows that there are, in fact, two successive, intersecting longitudinal divisions. These findings are in general agreement with the observations of Hus (1902) and contradict the assumption of the majority of workers that the first division leading to spore formation is transverse.

The stages of mitosis in the earlier divisions are difficult to obtain as comparatively few cells at this stage are found. In later divisions, however, all stages of mitosis could be followed. The available evidence shows clearly that there is no reduction division at any stage of spore formation in *Porphyra umbilicalis* var. *laciniata*.

In cells that are dividing longitudinally preparatory to spore formation, the interphase nucleus can be easily recognized. The nucleus appears as a very thin nuclear membrane within which there is a nucleolus, a considerable volume of non-staining material and usually one to seven stained bodies (Text-figs. 61, 62). The latter often appear as spherical to subspherical bodies of varying sizes just within the nuclear membrane (Text-fig. 61). These are interpreted as being heterochromatic segments of chromosomes. The number of these heterochromatic segments varies, probably with the stage of the kinetic nucleus. As the nucleus prepares to divide, these heterochromatic segments become more conspicuous one after another in succession, a fact which might account for the variation observed in different nuclei. More than one nucleolus is frequently present, two being commonly seen. Occasionally

TEXT-FIGS. 61–79. *Porphyra umbilicalis* var. *laciniata*. 61. Interphase nucleus in spore-forming cell showing six heterochromatic bodies at the periphery of the nucleus. 62. An interphase nucleus with five nucleoli and several heterochromatic bodies. 63. Spore mother-cell with early prophase nucleus. 64. Mid-prophase nucleus in spore mother-cell showing five chromosomes around the faintly stained nucleolus. 65. Spore mother-cell with late prophase nucleus, showing five chromosomes. 66. Interphase nucleus in a later cell generation in spore formation. 67–69. Early prophase nuclei, Fig. 68 that of nucleus stained with Schiff's reagent. 70. Mid-prophase nucleus with five chromosomes around the nucleolus. 71–73. Late prophase nuclei showing progressive shortening of the chromosomes. Fig. 73 that of nucleus stained with Schiff's reagent. 74. Mid-prophase nucleus showing small nucleolus. 75. Metaphase in side view. 76. Metaphase in polar view. 77, 78. Two stages in anaphase. 79. Telophase.

All figures × 1,800.

there may be more, as many as five having been observed (Text-fig. 62). These features are characteristic of cells that have not divided transversely. These cells have been studied both in surface view of the thallus and in sections.

Nuclei in early, mid-, and late prophase were also found in spore mother-cells undergoing the first division (Text-figs. 63–65; Pl. 1, Fig. 15). These divisions have been observed in surface view of whole mounts. The earliest prophase stage observed consisted of a nucleolus, faintly stained, surrounded by a number of strands of chromatin extending in all directions (Text-fig. 63). The number of strands could not be determined exactly as they seem to meet at various points rendering counting difficult. In mid-prophase, the nucleolus still persists, but only five chromosomes are now seen. These are distinct from one another and seem to be arranged around the nucleolus (Text-fig. 64). In late prophase the nucleolus is no longer seen. The five chromosomes become more or less condensed and isodiametric. They also move closer together (Text-fig. 65; Pl. 1, Fig. 15). Thus it appears reasonably certain that the chromosome number in cells that have not yet divided transversely is five, as seen in late prophase. It should be pointed out, however, that it is very difficult to establish whether the cells in consideration are going to divide transversely or longitudinally. The only way to ascertain this is by measuring the cells. The cells observed in this instance were about $20\mu \times 13\mu$. Therefore they are similar in size to the enlarged mother-cells of spores. Later stages of mitosis were not found for these early divisions.

In later divisions of spore-forming cells, however, all stages of nuclear division have been observed. In all these divisions, the process of mitosis has been the same. A detailed description of the process as seen in these later divisions is given below.

In early prophase, heterochromatic segments appear in the chromosomes and the nucleolus persists as a clearly defined, darkly stained body (Text-fig. 66). In subsequent stages of early prophase, the nucleolus loses its definite form and appears as a faintly stained central mass with a number of isolated stained bodies around it (Text-figs. 67, 68; Pl. 1, 16). These bodies are more deeply staining segments of the chromosomes. Subsequently, portions intervening between these segments become stainable and in such nuclei there is a number of chromatin strands. In mid-prophase, the chromatin strands become more definite and the number of such strands could be easily counted. They are found surrounding and arching over the nucleolus which appears somewhat diminished in size (Text-fig. 69; Pl. 1, Fig. 17). The number of these strands varies in different nuclei; it is most commonly seven or eight, but occasional nuclei may show five, six, or nine strands. Counts were made of the number of strands seen in this stage of the division from slides made from different thalli, collected at different times, the specimens being chosen quite at random. The slides examined included thalli stained in aceto-carmine, iron alum haematoxylin and Schiff's reagent. The results are given in Table 1.

TABLE 1

Counts of Chromatin Strands in Early and Mid-Prophase in Spore-Forming Cells

Slide no.	Stain	Number of nuclei examined	Number counted						
			5	6	7	8	9	10	Indefinite
215	Haematoxylin	42	15	3	9	10	5	—	—
216	,,	23	8	1	5	8	1	—	—
232	Feulgen	50	2	1	19	23	4	—	1*
50	Aceto-carmine	22	7	1	8	6	—	—	—

* Probably an earlier stage.

In a later stage of mid-prophase, only five chromosomes are seen surrounding the now feebly stained nucleolus. The chromosomes at this stage have a very characteristic appearance as they are arched over the nucleolus (Text-figs. 70, 74).

In late prophase, the nucleolus disappears completely and five slightly elongate chromosomes are seen surrounding a central space (Text-figs. 71, 72). Later, the chromosomes condense still further and even become subspherical (Text-fig. 73; Pl. 1, Fig. 18). In this stage the chromosomes are seen lying in slightly different focal planes.

In metaphase the chromosomes come closer together, and in side view form a transversely extended rod-like body (Text-fig. 75). In polar view, the five chromosomes are arranged in a crescent. They are so near to one another that in some instances they appear to form a continuous crescent (Text-fig. 76). The spindle could be seen in side view as a very fine fibrillar structure extending but a short distance on either side of the equatorial plate (Text-fig. 75).

At anaphase, two crescent-like groups of chromatids separate and move towards the poles (Text-fig. 77). The interzonal spindle is seen very clearly at this stage while the polar portions of the spindle are not apparent. As the anaphase proceeds and the chromosome groups move farther apart, the interzonal spindle becomes attenuated (Text-fig. 78).

In telophase, the chromosome plates condense into more or less crescent-like bodies and a hyaline area is organized around each (Text-fig. 79). The details regarding the later stages of mitosis are thus similar to those in vegetative cells.

Although the general pattern of mitosis is more or less uniform, the vegetative mitosis and the mitoses leading to the formation of both spermatia and spores nevertheless show differences in details, especially in the early and mid-prophases. In cells forming spores very early in prophase, there is an appearance of heterochromatic bodies which are not seen in vegetative mitosis. Moreover, the prophase is an extended phase of mitosis in spore-forming cells while in vegetative cells this is comparatively short. This does not mean,

however, that the prophase in vegetative mitosis is different, but only that the stages in the prophase of vegetative mitosis follow one another quickly so that the complete sequence has not become available. In the early prophase in spore-forming cells, the chromosomes show differential staining properties, some segments staining earlier and more deeply than others. This gives a false impression that there are more than five chromosomes in the nucleus. This phenomenon is more marked in spore-forming cells than in spermatium-forming cells. The nucleolus persists throughout the early and mid-prophase in both vegetative mitosis and the mitosis of spore-forming cells, but in the spermatium-forming cells there is a tendency for the nucleolus to disappear earlier, especially in the later divisions. Another difference between nuclei in the vegetative cells and those in reproductive cells relates to the number of nucleoli in interphase. In the reproductive cells there are often more than one nucleolus, commonly two, sometimes as many as five. When there are more than one nucleoli, they are usually smaller than the normal nucleolus. The presence of several nucleoli is probably referable to particular metabolic conditions in the reproductive cells.

The Problem of Fertilization

Spore-forming thalli were closely examined to ascertain whether there is a process of fertilization of the type described by Berthold (1882) and other workers. The facts of observation are briefly stated below.

1. Of more than fifty thalli examined, only two, one collected at Rhosneigr in April 1956, and the other collected at Port Erin in the Isle of Man in April 1957, showed 'fertilization canals' as described by Berthold (1882). In all the other thalli, though spore formation was normal, there was no trace of a 'fertilization canal'.

2. On the surface of several thalli genuine spermatia were seen, but these showed no connexion whatever with the contents of the cell below. Often these were found in regions where spore formation had already proceeded to an advanced stage.

3. Beside the spermatia, there were other spherical cells of an unknown nature on the surface of the thallus. These cells had inter-phasic nuclei, were of variable sizes, and had no recognizable chromatophore and so were different from genuine spermatia which possess nuclei in late prophase, include a definite, pale chromatophore and are of uniform size.

4. Where 'fertilization canals' were seen, these either ended blindly in the mucilaginous sheath of the thallus or else extended into the intercellular layer between the cells of the thallus.

A consideration of the foregoing observations shows that practically no evidence of fertilization of the type described by Berthold and other earlier workers was obtained in *Porphyra umbilicalis* var. *laciniata*.

A second view regarding sexual reproduction in *Porphyra* is that the spores and spermatia are both gametes and that these fuse in pairs after liberation (Derbes and Solier, 1856; Koschtsug, 1872; Knox, 1926). In order to test

this, observations were made on liberated spores and spermatia brought together on slides. In an extended series of observations, no evidence of fusion was obtained.

It would seem that the evidence actually obtained for sexual reproduction in *Porphyra* is very slender and even doubtful. Until more conclusive evidence is forthcoming, the problem of sexual reproduction must remain an open question. The resolution of this problem is intimately connected with the nature of the spermatium. This is generally regarded as the male gamete, though, as pointed out above, there seems to be little evidence to regard it as such. An attempt was therefore made to see if the spermatia would germinate and a series of cultures were set up for this purpose. They failed to germinate and disintegrated after a few days. Hence it is not possible to state the nature of the spermatia at this stage.

Cytology of the *Conchocelis*-Phase

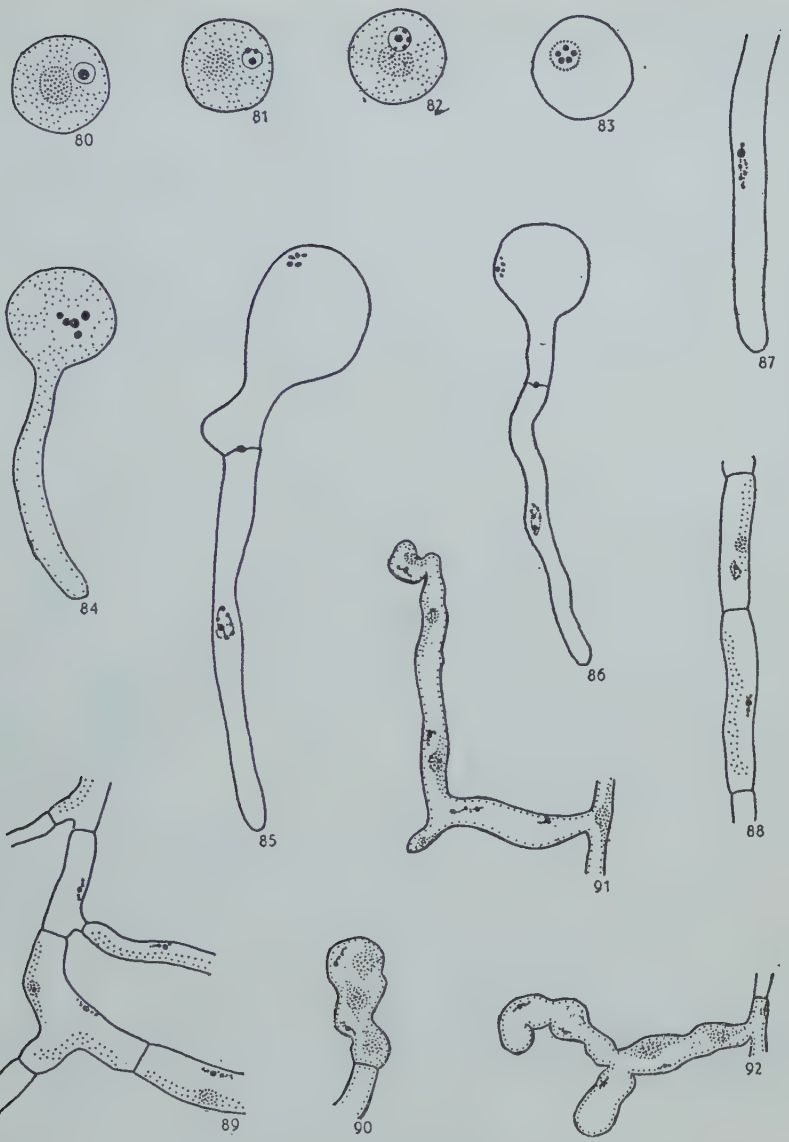
Germination of the spore and the development of the vegetative Conchocelis-*phase*

The liberated spore is spherical and has a well-defined chromatophore and a nucleus in interphase (Text-fig. 80). The amoeboid movement of the mature spore, described by earlier observers was not seen. But spores which are not developed fully, if liberated under artificial conditions in culture, are often of an irregular shape at the time of liberation and such spores show an amoeboid change of shape. This amoeboid change of shape, however, stops once the spore has assumed a spherical shape.

When cultured on a glass slide, the spore germinates in 2–5 days, by putting forth a tubular prolongation which may grow out in any direction. Generally this prolongation is creeping, but it may be erect, especially when a large number of spores are germinating in a restricted space. The incidence of germination is fairly high, about 60 per cent. of the spores germinating in 5 days.

On shells, the spore forms a tubular prolongation which at first lies on the surface of the shell, but soon turns downwards and pierces the shell. Thenceforward, the growth is within the shell but parallel to its surface. The pattern of growth of this shell-inhabiting phase, which is identical with *Conchocelis rosea* Batters and named the *Conchocelis*-phase by Drew (1949), is very characteristic and is presumably the result of growth in a special environment.

During the germination of the spore, heterochromatic bodies can be observed in the nucleus, as in the mitoses during formation of reproductive cells on the leafy thallus. Two to five such bodies were observed in the nuclei of spores that had not put forth germ-tubes (Text-figs. 81, 82). During this stage in germination, the spore enlarges and becomes vacuolated and the nucleus is pushed against the spore-wall and so appears flattened. In surface view, the nucleus occupies a rounded to oval area, while in side view it appears as a narrow elongated body. In a number of spores, late prophase



TEXT-FIGS. 80–92. *Porphyra umbilicalis* var. *laciniata*. 80. Spore with interphase nucleus. 81, 82. Spores with nuclei in which heterochromatic bodies are appearing. 83. Germinating spore with nucleus in late prophase showing five chromosomes. 84. Germling with germ-tube not separated by cross wall, showing late prophase nucleus with five chromosomes. 85, 86. Two more germlings with germ-tube separated by a cross-wall. Nucleus in the spore is in late prophase. Those in tube show characteristic structure found throughout the vegetative *Conchocelis*-phase. 87. End cell of a young *Conchocelis* filament, showing elongated nucleus. 88, 89. Filaments from the vegetative *Conchocelis*. 90–92. Stages in formation of 'fertile cell rows', showing variously swollen filaments with several nuclei and pyrenoids.

Figs. 80–87: ×900; Figs. 88–92: ×450.

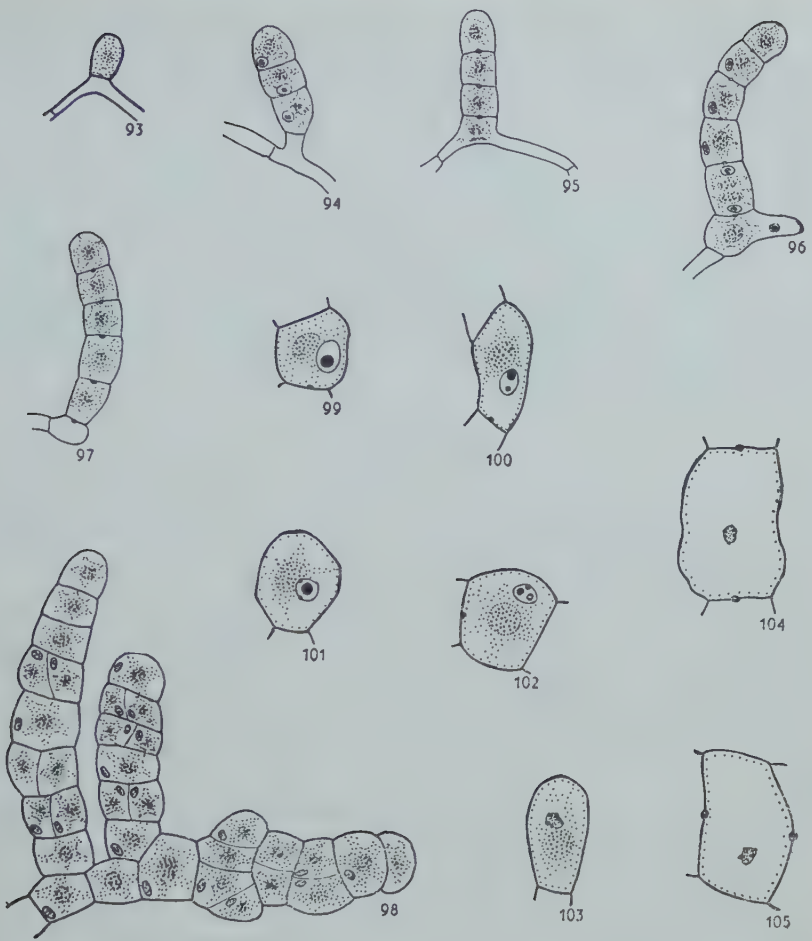
nuclei were seen and these had five chromosomes which were more or less isodiametric (Text-fig. 83; Pl. 2, Fig. 1). The spore often forms its germ-tube before the nucleus has completed division (Text-fig. 84; Pl. 2, Figs. 2, 3). The germ-tube is at first continuous with the spore, but after nuclear division one of the daughter nuclei passes into it and a partition wall is laid down. This wall invariably shows a prominent pit-connexion (Text-figs. 85, 86; Pl. 2, Figs. 4, 5). After one germ-tube has been established, the nucleus left in the spore may divide again and another germ-tube may be formed. In the germ-tube, and in the segments of the *Conchocelis*-phase in general, the nucleus is usually elongated and appressed to the wall of the filament and shows a nucleolus and several heterochromatic bodies inter-connected in various ways by thin strands of stainable chromatin (Text-figs. 85–89).

Development and cytology of the 'fertile cell rows' and 'plantlets'

Sooner or later, the *Conchocelis*-phase develops what have been referred to as 'fertile cell rows'. The mode of their development is similar in both free-living and shell-inhabiting filaments and is identical with that described by Drew (1954*a*). In the present cultures this was followed easily in free-living filaments. Some branches of the filaments become swollen irregularly and show a dense aggregation of chromatophore pigments. These filaments may be simple (Text-fig. 90) or branched in various ways (Text-figs. 91, 92; Pl. 2, Fig. 7). In these swollen filaments one to several chromatophores are formed and in these pyrenoids appear. There are one to several nuclei in these filaments, each generally associated with a chromatophore. Cross walls are then formed separating these. The 'fertile cell rows' that are thus formed are filaments of varying lengths and degrees of branching and are usually terminal in position. The number of cells comprising a 'fertile cell row' is variable. One-celled branches have been seen at one extreme, and on the other were long, branched filaments of several cells (Text-figs. 93–97; Pl. 2, Fig. 6). The cells possess large, axile, stellate chromatophores with prominent pyrenoids, similar to those of the leafy phase.

There is evidence that the 'fertile cell rows' are capable of growth by the division of their cells (Text-fig. 96; Pl. 2, Fig. 6). The alignment of the nuclei after division resembles that found during the division of a vegetative cell in the leafy phase.

In the 'fertile cell rows', the interphase nucleus is similar to that of the leafy phase. It has a prominent nucleolus, surrounded by a non-staining nuclear material and a very thin nuclear membrane (Text-fig. 99). In very early prophase, one to three heterochromatic bodies can be observed (Text-figs. 100–2; Pl. 2, Fig. 9). Mid-prophase nuclei were seen in two cells in a section stained with brazilin and in one cell in a squash preparation stained with Schiff's reagent. In two of these cells, there were six to seven stained chromatin segments surrounding the nucleolus which was itself feebly stained (Text-figs. 103–4; Pl. 2, Figs. 10, 11). In the third cell, there were five chromosomes surrounding the nucleolus (Text-fig. 105). Later stages of



TEXT-FIGS. 93–105. *Porphyra umbilicalis* var. *laciniata*. 93–97. Various stages in formation of 'fertile cell rows'. In Fig. 96 one cell shows division into two in a transverse plane. 98. A portion of a plantlet, showing cells divided by thin vertical walls. 99. Cell from 'fertile cell row' with interphase nucleus. 100–2. Cells from 'fertile cell rows' showing hetrochromatic bodies in nuclei. 103–4. Nuclei in prophase with six to seven stained chromatin segments, in cells of 'fertile cell rows'. 105. Cell from 'fertile cell row' showing nucleus in late prophase with five chromosomes.

Figs. 93–98: ×450; Figs. 99–105: ×900.

mitosis were not seen. The available information regarding the nuclear division in the *Conchocelis*-phase shows that it is similar to that in the leafy phase. The chromosome number is five, as seen in late prophase, both in the vegetative *Conchocelis*-phase and in the 'fertile cell rows'.

In shells in which the *Conchocelis*-phase has been growing for over a year, 'plantlets' of the type described by Drew (1954*a*) were found (Pl. 2, Fig. 8), but no spore liberation. These 'plantlets' consisted of rosettes of filaments

with cells similar to those of the 'fertile cell rows'. The branches of the filaments consisted of either a single linear series of cells or, frequently, the cells were divided into two in a vertical plane (Text-fig. 98). The nuclei showed a very early prophase condition. Later stages of mitosis, however, were not found.

Although liberation of conchospores from the 'fertile cell rows' has been recorded by a number of workers in recent years, in the writer's cultures no spore liberation occurred during observations extending over more than a year. It is possible that in *Porphyra umbilicalis* var. *laciniata*, such a spore liberation does not take place, there being a probable, alternative method of re-establishment of the leafy phase. It is in this connexion that the occurrence of the 'plantlets' is significant.

Discussion

In this study, the author's aim was to investigate the life-history of *Porphyra umbilicalis* var. *laciniata*, with special reference to its cytology. As a result, the following points of major interest have been established.

1. Since no evidence of diploid nuclei at any stage in the life-history has been obtained, the correctness of the reports of earlier workers on the occurrence of sexual reproduction is a matter of considerable doubt; it is certainly one that should be accepted with critical reserve.

2. Contrary to views that have been held, the nucleus of *Porphyra* shows many of the organizational features and features of mitosis found in higher plants.

3. Although the nuclei of *Porphyra* are very small, it has nevertheless been possible to obtain evidence of their variable metabolic status. This can usually be related to the metabolism of the cell and the phase of development.

4. Although the existence of two quite distinct somatic phases has now been demonstrated, viz. the leafy phase and the *Conchocelis*-phase, both occurring abundantly in nature, the mode of origin of the former from the latter is still an obscure and unresolved problem.

5. The present critical study has made possible a reassessment of the observations and conclusions of earlier workers, both in respect of life-history and cytology.

6. Notwithstanding the effort that has gone into the present and previous studies, the life-history of *Porphyra* is still not completely understood. There are gaps to be filled in the sequence of somatic phases and the role of the different reproductive bodies has to be established with certainty. Further work may profitably be concerned with (*a*) the validity of Dangeard's observation of genuine monospores; (*b*) the role of spermatia in the life-history; (*c*) the mode of establishment of the leafy phase from the *Conchocelis*-phase; (*d*) the presence or absence of a sexual cycle in the life-history of this alga.

A more detailed consideration of the points mentioned above is made in the succeeding pages.

The evidence for sexual reproduction

The chromosome number in *P. umbilicalis* var. *laciniata* has been ascertained in the vegetative cells of the leafy phase, the spermatium-forming cells, the spore mother-cell, subsequent cell generations during spore-formation, liberated spores, germinating spores, the segments of the vegetative *Conchocelis*, and in the 'fertile cell rows' of the *Conchocelis*-phase. In all these stages the chromosome number is five, showing that *Porphyra* is haploid throughout. In view of this, it is interesting to reconsider the earlier reports of sexual reproduction in *Porphyra*. Berthold (1882), Joffe (1896), Ishikawa (1921), Grubb (1924), Dangeard (1927), Kunieda (1939), Magne (1952), and Tseng and Chang (1954–5), all believed in or produced evidence for 'fertilization' of an unmodified cell of the thallus. The evidence included observation of a 'spermatium' lodged on the surface of the thallus and connected in various ways with a cell of the thallus, and the presence of 'male nuclei' at various positions either in the 'fertilization tube' or in the 'female cell'. The present writer did not see any evidence of a genuine spermatium effecting fertilization of an unmodified cell. True, a number of spermatium-like cells were seen on the surface of the thallus, but these proved to be different from spermatia. Moreover, if a process of fertilization at all similar to that in Florideophycidae is expected to occur in *Porphyra*, then the fusing gametic nuclei should be in a characteristic state. The nucleus of the female gamete would be in interphase but that of the spermatium would be *in late prophase as seen in the liberated spermatium*. It is not possible to conceive of the late prophase nucleus changing into an interphase nucleus, unless the spermatium had divided after liberation. But for this, there is no evidence.

All these workers were following Berthold in supposing that there is necessarily a form of sexual fusion as in the Florideophycidae. For Berthold, working in the early days following the discovery of the process of fertilization in the Florideophycidae, it is understandable that the most obvious interpretation of the structures seen by him was that they were stages of fertilization. The fact that *Porphyra* and allied forms were related to the Florideophycidae in regard to pigmentation of the chromatophore, nature of the reserve material, nature of the cell wall and the absence of motile reproductive elements, could have easily led any one to expect a mode of sexual reproduction also similar to that in the Florideophycidae.

Dangeard (1927) and Tseng and Chang (1954–5) also sought to present evidence of reduction division prior to formation of spores. The figure given by Dangeard shows very little, although he claims to have demonstrated reduction division. Tseng and Chang have given a drawing and a photograph of what they consider to be 'synapsis'. These show five double-stranded chromosomes, but there is evidence in the present work that in well differentiated material, a double-strand structure may be seen even in chromosomes of mitoses leading to the formation of spermatia.

Magne (1952) differs from all these workers in maintaining that there is no

reduction division immediately following fertilization. The only prophasic nucleus he has drawn is that of a cell derived by the transverse division of what is regarded as the fertilized 'carpogonium', and this shows about six elongated structures claimed to be the chromosomes of the diploid nucleus. Magne claims to have seen this condition in all stages of spore formation. Apart from the fact that the number of structures seen in his figure is six and not eight, there is a close similarity between his figure and the mid-prophase nuclei observed by the present writer. The elongated nature of the structures observed by Magne suggest rather that they are stained chromatin segments of the earlier stages of prophase and so do not represent the actual number of chromosomes.

Thus, in the writer's opinion, the observations of the previous workers were wrongly interpreted, probably because they were all predisposed to believe in Berthold's report of fertilization in the genus. There is no evidence of fertilization, at least as far as *P. umbilicalis* var. *laciniata* is concerned, and there is no diploid condition prior to formation of spores. The so-called 'carpospores' are not homologous with the carpospores of the Florideophycidae.

One other point of importance may be made here. These several workers also followed Berthold in assuming that the first division in a spore mother-cell was transverse. Careful observations on both whole mounts and sections of the thallus show that the first division leading to spore-formation is longitudinal, as pointed out already by Hus (1902), and that this division is attended by definite phenomena associated with metabolic changes in the nucleus: (1) Prior to spore formation, vegetative cells destined to form spores enlarge and this can be seen in surface view. (2) Two intersecting longitudinal walls are laid down in these cells and these walls also can be seen in surface view. (3) The nuclear divisions in these enlarged spore mother-cells are in advance of cytokinesis, so that cells with four nuclei may be just commencing cytoplasmic cleavage. (4) The interphase nuclei of these cells show heterochromatic bodies close to the nuclear membrane, unlike nuclei of vegetative cells. The last two features are seen in enlarged cells of the thallus, both in surface view and in sections.

In view of the fact that the first division leading to the formation of spores is longitudinal, any evidence for reduction division should be sought for in this division and not in the transverse division, which is, in fact, the third division in the sequence of formation of spores.

There are also other arguments against considering the spores as carpospores. (1) The 'carpospores' and the spermatia are both formed in the same manner, by repeated division of a mother-cell. This suggests that the two are homologous structures. It is unlikely that a gamete and a spore should be formed in identical ways. The cytological evidence here presented emphasizes the similarity in their mode of formation. (2) The spores are produced over large areas of the thallus and if they are carpospores, one has to assume that every cell in this area is a potential gamete and that every one of them is

fertilized. This, again, is very improbable. (3) There are records in the literature (Janczewski, 1873) of formation of both spores and spermatia from two halves of the same mother-cell. This could happen only if the two are homologous structures.

The process of mitosis in Porphyra

In the present investigation the vegetative mitosis was examined in considerable detail as being likely to enable one to assess more adequately the nature of the nuclear divisions in various critical stages of the life-history. The writer considers that the nucleus is well organized and the process of mitosis quite normal and that it is not 'intermediate between mitosis and amitosis' as was stated by Ishikawa (1921). This supports the view of Dangeard (1927) that the nuclear divisions in vegetative cells of *P. umbilicalis* and *P. umbilicalis* f. *linearis* are normal.

There are, however, two features in the vegetative mitosis of *Porphyra* which are interesting. In early prophase, there are a number of stained chromatin segments and these are not precisely indicative of the chromosome number; rather, they are to be recognized as segments of the chromosomes which stain more deeply and earlier than the remaining parts. Subsequently, the intervening portions of the chromosomes become stainable and the entire chromosomes become recognizable. A comparison may be made with the situation in *Spirogyra* spp. recorded by Godward (1954). She uses the term 'stained blocks' to describe the early staining segments of the chromosomes.

The second characteristic feature in the mitosis in *Porphyra* concerns anaphase. In early anaphase, the chromatids separate as two crescent-shaped groups and an interzonal spindle develops and elongates as the two groups of chromatids move apart. The interpretation of the crescent-shaped groups is uncertain. The shape is so constant that it suggests that the groups may now be acting as a unit, in which case comparison could perhaps be made with one of the polycentric 'chromosomes' described for *Spirogyra* by Godward (1954). In the case of *Porphyra*, however, cohesion of the components of the anaphase group could only be inferred, but not demonstrated owing to their very small size.

In the cells forming spermatia and spores, the sequence of events in mitosis is similar to that in vegetative cells except for differences in the interphase nucleus and in the prophase, which is of more extended duration in the spore-forming cells.

The appearance of heterochromatic bodies in the interphase of spore-forming cells suggests that the metabolic relationships are very different from those in vegetative cells. In the later stages of spore formation, there is further evidence of specific metabolism in the frequent presence of more than one nucleolus. The number of nucleoli observed is mostly two, but more have been seen, and in a few instances a maximum of five nucleoli are seen. It is possible that, in a primitive alga like *Porphyra*, all the chromosomes are capable of organizing nucleoli, and, if so, each of them would tend to form a

nucleolus by itself. In a prolonged interphase, all the nucleoli formed would ultimately coalesce to form a single nucleolus; but if the interphase is short, as is likely during the later divisions in spore-formation, the different nucleoli may remain independent or fuse to varying degrees, so that more than one nucleolus may be seen at this stage.

Of the earlier workers Ishikawa (1921) appears to have noticed these features of the interphase nucleus in spore-forming cells. His figures certainly indicate this; but he did not appreciate this point, as he considered the nucleus to be 'fragmenting'. This assumption is evidently due to his having mistaken the nucleolus for the entire nucleus. He did not see the unstained nuclear material and the nuclear membrane surrounding the nucleolus, as these structures are scarcely visible and are to be seen only in well-differentiated material. This failure to recognize the nuclear membrane has led to his assumption that the heterochromatic bodies and the extra nucleoli seen in interphase are 'fragments' of the nucleus. From this he goes on to state that there is a 'primitive form of meiosis' in *Porphyra*.

The life-history of Porphyra

The facts of the life-history of *P. umbilicalis* var. *laciniata* established by the present study are: (1) there are two somatic phases of contrasting habit and structure, namely, the leafy phase and the *Conchocelis*-phase; (2) the leafy phase produces spores and spermatia; (3) spores germinate to produce the *Conchocelis*-phase, the function and fate of the spermatia being still unknown; (4) the *Conchocelis*-phase gives rise to 'fertile cell rows' and ultimately to 'plantlets', but unfortunately the process of establishment of the leafy phase from the *Conchocelis*-phase could not be ascertained in the limited time at the disposal of the writer. Cultures of the *Conchocelis*-phase were, however, maintained over a period of eighteen to twenty months. While all the cultures produced 'fertile cell rows' and some produced 'plantlets', none of them liberated conchospores, in spite of various attempts to induce their liberation. This is all the more striking in view of the fact that various workers in Japan and elsewhere have been able to observe not only the liberation of conchospores but also their germination and subsequent development into the leafy phase (Kurogi, 1953*a*, 1953*b*; Graves, 1955; Suto, 1954; Suto *et al.*, 1954; Takeuchi *et al.*, 1954; Tseng and Chang, 1954–5; Yamasaki, 1954*b*). Drew (1954*a*, 1954*b*, 1955) found 'plantlets' developing in her cultures, but no spore liberation. Dangeard has always maintained (1931, 1933, 1954*a*, 1954*b*) that the *Conchocelis*-phase was protonemal in nature and that the leafy phase arises from this as 'pluricellular buds'. Ogata (1955) observed uniseriate upright plants on the pieces of calcite in which he raised his cultures. He assumed that these had arisen from spores produced on the 'fertile cell rows'.

'Plantlets' have also been observed in the present cultures and these occurred both on the surface of the shells and in the interstices between the lamellae of the shells used for raising the cultures. The structure of the 'plantlets' recalls the description of the conchospore-forming phase by Tseng and

Chang (1954–5). They state that the cells of the 'fertile cell rows' undergo a final division before these round up to form two conchospores per cell. In the 'plantlets' of the present culture, most segments of the filamentous branches are divided by very thin vertical walls. These might correspond to the final divisions in the 'fertile cell rows' described by Tseng and Chang, but the writer has not seen them liberated as conchospores.

What really happens in the 'plantlets' of *P. umbilicalis* var. *laciniata* must at present remain largely conjectural. One possibility that suggests itself is that these 'plantlets' might eventually grow out as the leafy phase without the production of conchospores, a possibility which would be in conformity with Dangeard's view. Alternatively, given the right environmental conditions, the cells may give rise to conchospores which on liberation may give rise to the leafy phase. In this connexion it should be appreciated that the interphase nuclei of the 'fertile cell rows' show similarity to those of spore-forming cells of the leafy phase, thus indicating a similarity in the metabolic state of these nuclei. It is possible that higher temperature and other factors of environment govern the formation and liberation of spores from the *Conchocelis*-phase. It should be remembered that the Japanese and Chinese workers have been working in lower latitudes, where there is a higher average temperature throughout the year. Also, these workers have observed that the spore liberation in the *Conchocelis*-phase takes place at higher temperatures, the optimum temperature being about 25° C. This would account for the abundant spore liberation reported by these workers and the absence of spore liberation in the cultures grown by Drew and the present writer in England. The possibility should not be overlooked that the life-history of *Porphyra* may also present different features under different environmental conditions as in several other marine algae such as *Ectocarpus*.

There is, finally, the fate of the spermatia to be considered. It has been shown that these do not effect fertilization of a cell of the thallus, as assumed by earlier workers. Efforts to induce their fusion with liberated spores have failed. Attempts to induce them to germinate have also failed. But the facts remain that spermatia are formed most abundantly, that the cytology of their formation is normal and consistent throughout, and that, when liberated, they have a nucleus in a characteristically late prophase condition similar to that of the spermatia of the Florideophycidae. These features may indicate that the spermatia are indeed of the nature of true spermatia, but their fusion with another gamete has yet to be observed. It is possible that the spores are really the second type of gamete and that these develop apomictically. If, on the other hand, the spermatia are a second type of spores, further work is necessary to ascertain if, and how, they germinate and into what structures they develop. This can be done only by means of aseptic culture under controlled conditions.

The above discussion indicates that the life-history of *Porphyra* still remains incompletely understood. Until recently, attention was concentrated only on the mode of formation of spores and their subsequent development.

But the essential problem that has yet to be solved relates to the nature of the spermatia and the role they play in the life-history. The question of whether the absence of a diploid phase in the life-history is a primitive or a degenerate feature has also to be solved. Only then a complete picture of the life-history of *Porphyra* can be obtained.

Acknowledgements

The writer is highly indebted to the late Dr. K. M. Drew-Baker, who suggested this problem and gave valuable guidance during the course of this study. He is greatly indebted to Prof. C. W. Wardlaw, Miss A. P. Wylie, and Prof. I. Manton for kind criticism and advice in the preparation of this paper. His thanks are due to Mr. E. Ashby and Mr. G. Barker for helping with the photography. This investigation was carried out during the tenure of a Fellowship under the Colombo Plan. The writer is grateful to the Ministries of Education, Governments of Madras and India, who sponsored him for the Fellowship.

Literature Cited

Arasaki, S., 1954: On the Life-history of *Porphyra tenera* Kjellm. Rijaku (Science), **24,** 152–3.

Berthold, G., 1882: Die Bangiaceen des Golfes von Neapel und der angrenzenden Meeresabschnitte. Fauna u. Flora des Golfes Neapel, etc., **8,** 1–28.

Chadefaud, M., 1952: Sur le cycle sexuel des organismes eucaryotes et son evolution. Rev. Sci., Paris, **90,** 49–57.

Coleman, L. C., 1938: Preparation of Leuco-basic Fuchsin for Use in the Feulgen Reaction. Stain Tech., **13,** 123–4.

Dangeard, P., 1927: Recherches sur les *Bangia* et les *Porphyra*. Le Botaniste, **18,** 183–244.

—— 1931: Sur le development des spores chez quelques *Porphyra*. Trav. Cryptogam. ded. à L. Mangin, 85–96.

—— 1933: *Traité d'Algologie*, Paris.

—— 1954*a*: Introduction à la serie 38. Le Botaniste, **38,** 5–14

—— 1954*b*: Contribution à la connaissance du cycle evolutif des Bangiacees. Huitième Congrès International de Botanique, Paris. Rapports et communications à la Section 17, 76–79.

Derbes, A., et Solier, A. J. J., 1856: *Mémoire sur quelques points de la physiologie des algues*, Paris.

Drew, K. M., 1934: Contributions to the Cytology of *Spermothamnion turneri* (Mert.) Aresch. I. The Diploid Generation. Ann. Bot. Lond., **48,** 549–73.

—— 1949: *Conchocelis*-phase in the Life-history of *Porphyra umbilicalis* (L.) Kütz. Nature, **164,** 748.

—— 1954*a*: Studies in the Bangioideae. III. The Life-history of *Porphyra umbilicalis* (L.) Kütz. var. *laciniata* (Lightf.) J.Ag. A. The *Conchocelis*-phase in culture. Ann. Bot., N.S., **18,** 183–211.

—— 1954*b*: Life-history of *Porphyra*. Nature, **173,** 1243.

—— 1955: Life-histories in the Algae, with Special Reference to the Chlorophyta, Phaeophyta and Rhodophyta. Biol. Rev., **30,** 343–90.

—— 1956: Reproduction in the Bangiophycidae. Bot. Rev., **22,** 553–611.

Feldmann, J., 1952: Les Cycle de reproduction des algues et leurs rapports avec la phylogenie. Rev. Cytol., **13,** 1–49.

Godward, M. B. E., 1954: The 'diffuse' Centromere or Polycentric Chromosomes in *Spirogyra*. Ann. Bot., N.S., **18,** 143–56.

Graves, J. M., 1955: Life-cycle of *Porphyra capensis* Kutz. Nature, **175,** 393.

GRUBB, V. M., 1924: Observations on the Ecology and Reproduction of *Porphyra umbilicalis* (L.) J.Ag. Rev. Algol., **1**, 223–34.

HUS, H. T. A., 1902: An Account of the Species of *Porphyra* found on the Pacific Coast of North America. Proc. California Acad. Sci., III. Bot., **2**, 173–241.

ISHIKAWA, M., 1921: Cytological Studies on *Porphyra tenera* Kjellm. Bot. Mag. Tokyo, **35**, 206–18.

JANCZEWSKI, E., 1873: Études anatomiques sur les *Porphyra*, etc. Ann. Sci. Nat., V. Bot., **17**, 241–60.

JOFFÉ, R., 1896: Observations sur la fécondation des Bangiacees. Bull. Soc. Bot. France, **43**, 143–6.

KNOX, E., 1926: Some Steps in the Development of *Porphyra naiadum*. Publ. Puget Sound Biol. Stat., **5**, 125–35.

KOSCHTSUG, C., 1872: Entwicklungsgeschichte von *Callithamnion daviesii* Lyngb. und *Porphyra laciniata* Ag. Abstract. bot. Jahresber., **1** (1874), 13.

KUNIEDA, H., 1939: On the Life-history of *Porphyra tenera* Kjellm. J. Coll. Agric., Tokyo Imp. Univ., **14**, 377–405.

KUROGI, M., 1953*a*: Study of the Life-history of *Porphyra*. I. Germination and Development of Carpospores. Bull. Tohoku Reg. Fish. Res. Lab., **2**, 67–103.

—— 1953*b*. On the Liberation of Monospores from the Filamentous Thallus (*Conchocelis*-phase) of *Porphyra tenera* Kjellm. Ibid., **2**, 104–8.

KYLIN, H., 1946: Über die Sporenkiemung bei *Bangia* und *Porphyra*. Kungl. Fysiogr. Sallsk. Lund Forh., **16** (3), 1–4.

MAGNE, F., 1952: La Structure du noyau et le cycle nucleaire chez le *Porphyra linearis* Greville. C.R. Acad. Sci., **234**, 986–8.

OGATA, E., 1955: Perforating Growth of *Conchocelis* in Calcareous Matrices. (Date of publication and name of Journal not on reprint seen, but received for publication, 12 Dec. 1955.)

ROSENVINGE, L. K., 1909: The Marine Algae of Denmark, Part I. Rhodophyceae. I. Bangiales and Nemalionales. Dansk. Vid. Selsk. Skrift., **7**, Mat.-nat. Afd. 7 (1).

SUTO, S., 1954: On the Life-history of *Porphyra Tenera*. 3. Bull. Jap. Soc. Sci. Fish., **20**, 494–6.

—— and Fujiyama, T., 1952: On the Life-history of *Porphyra tenera*. Ibid., **17**, 1–4.

—— MARUYAMA, T., and UMABAYASHI, O., 1954: On the Shedding of Spores from the *Conchocelis*-phase of *Porphyra tenera* Cultured in Shells of Bivalves. Ibid., **20**, 490–3.

TAKEUCHI, T., MATSUBARA, M., SHITANAKA, M., and SUTO, S., 1954: On the Shedding of Spores from Cultured *Conchocelis*-phase of *Porphyra tenera* Set in Sea. Ibid., **20**, 487–9.

TSENG, C. K., and CHANG, T. J., 1954–5: Studies on the Life-history of *Porphyra tenera* Kjellm. Sci. Sinica, **4**, 375–98. (Date unknown. There is a footnote saying that the paper is a combination of two papers published in Chinese in Acta Botanica Sinica, **3** (1954), 287, and **4** (1955), 27.)

—— 1955: Studies on *Porphyra* III. Sexual Reproduction of *Porphyra*. Acta Bot. Sinica, **4**, 153.

—— 1956: Conditions of *Porphyra* Conchospore Formation and Discharge and Discharge Rhythm. Ibid., **5**, 33–35.

YAMASAKI, H., 1954: Studies on the Ecology of the *Conchocelis*-phase of *Porphyra tenera* Kjellm. I. Bull. Jap. Soc. Sci. Fish., **20**, 442–6.

—— 1954*b*: Studies on the Ecology of the *Conchocelis*-phase of *Porphyra tenera* Kjellm. II. Shedding and Fixing of Spores. Ibid., **20**, 447–50.

EXPLANATION OF PLATES

PLATE 1

FIGS. 1–18. *Porphyra umbilicalis* var. *laciniata*. 1. Early prophase in vegetative cell, with seven stained chromatin segments. 2. Mid-prophase with five chromosomes surrounding the nucleolus, four only seen in view. 3. Late prophase with five chromosomes. 4. Metaphase. 5, 6. Anaphase showing the interzonal spindle. 7. Telophase. 8. Spermatium-forming cell with nucleus in very early prophase. 9. Spermatium-forming cell, with nucleus in early prophase showing stained chromatin segments. 10. Mid-prophase with five chromosomes,

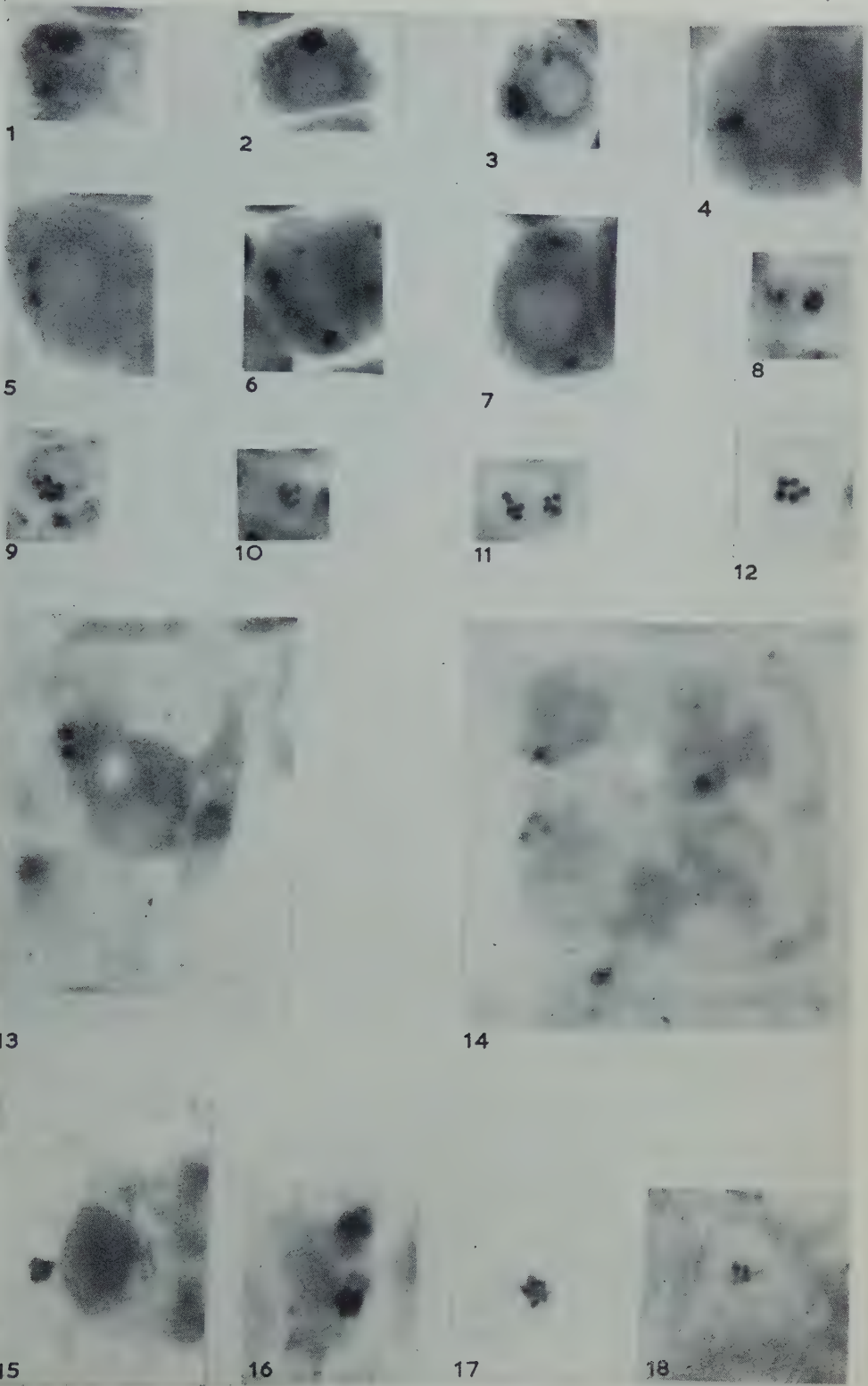
showing double strand structure. 11. Late prophase in spermatium-forming cell. 12. Prometaphase in spermatium-forming cell. 13. Spore mother-cell with three of four nuclei and a single chromatophore and undivided pyrenoid. 14. A spore mother-cell with four nuclei and showing commencement of cytoplasmic cleavage in both longitudinal and transverse planes. 15. Spore mother-cell with nucleus in late prophase showing five chromosomes. 16, 17. Spore-forming cell with nucleus in early prophase. 18. Spore-forming cell with nucleus in late prophase

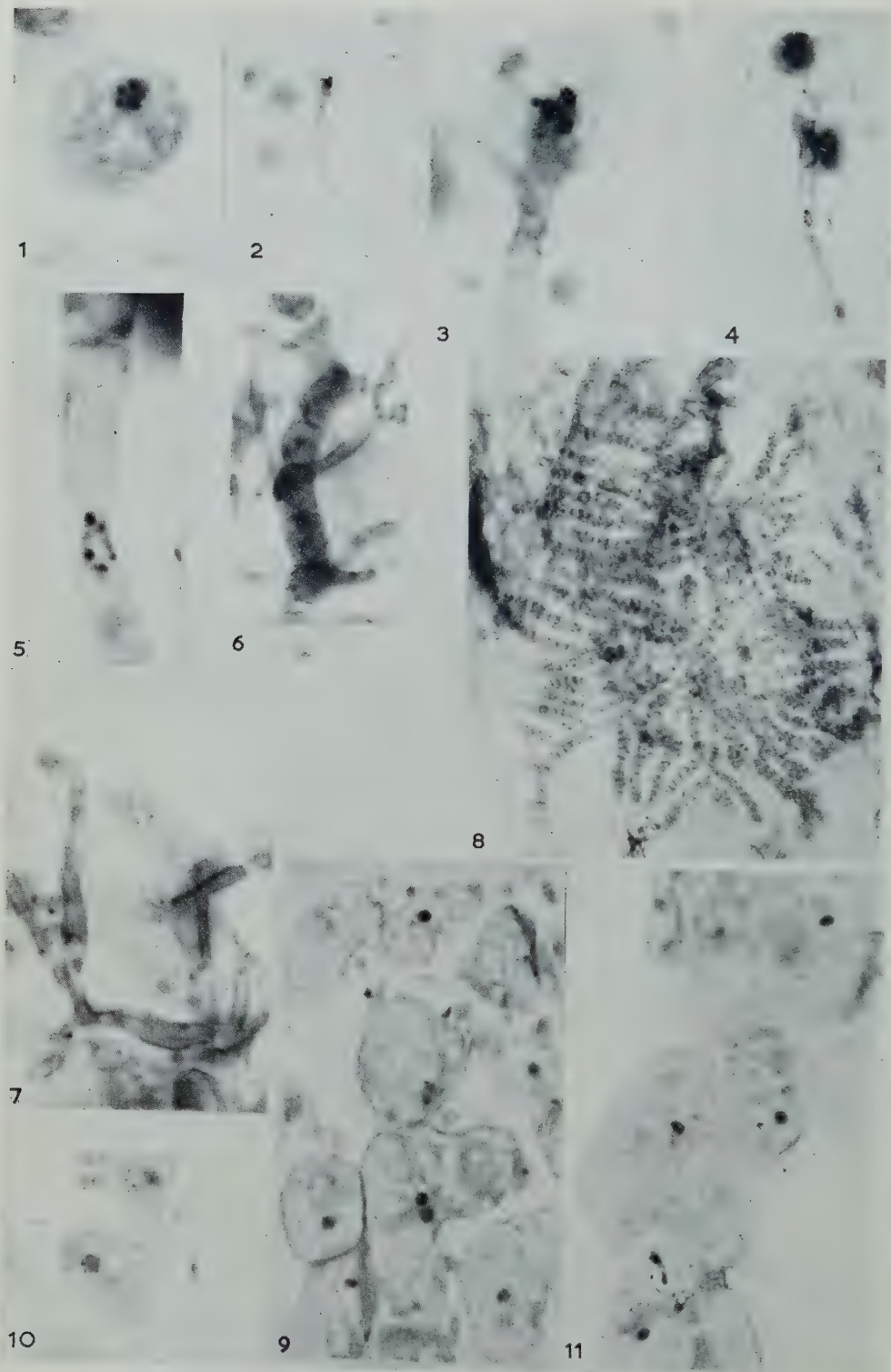
Figs. 1–11, 13, 14: ×1,660; Fig. 12: ×3,000; Figs. 15–18: ×1,960

PLATE 2

Figs. 1–11. *Porphyra umbilicalis* var. *laciniata*. 1. Spore showing nucleus in late prophase with five chromosomes. 2. One-celled germling with a nucleus in late prophase. 3. The same, showing detail of the nucleus. 4. Two-celled germling. 5. Germ-tube of the same, with nucleus of characteristic structure. 6. A 'fertile cell row'. The second cell from the bottom shows division. 7. A swollen and branched filament of the *Conchocelis*-phase, prior to formation of the 'fertile cell row'. 8. A 'plantlet'. 9. Cells of a 'fertile cell row' showing nuclei in interphase. 10. A 'fertile cell' with early prophase nucleus showing seven stained chromatin segments around the nucleolus. 11. Cells of a 'fertile cell row' with early prophase nuclei

Figs. 1, 3, 5: ×1,960; Figs. 2, 4, 6, 7: ×490; Fig. 8: ×100; Figs. 9–11: ×980


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18



V. KRISHNAMURTHY

The Analysis of Correlative Growth in the Etiolated Oat Seedling in Relation to Carbon Dioxide and Nutrient Supply

BY

C. L. MER

(*Research Institute of Plant Physiology, Imperial College of Science and Technology, London, S.W. 7*)

With nine Figures in the Text

Abstract

To overcome the reduced extension growth of the coleoptile which occurs when oats are grown in air enriched with 5 per cent. CO_2, plants have been provided with nutrients via the roots. 2 per cent. sucrose, glucose or mannitol so applied further promoted the mesocotyl and further depressed the coleoptile. Root growth was also depressed.

To induce promotion of coleoptile growth by externally applied sucrose, seedlings were heated in darkness at 40° C. for 3 hours so restricting selectively the growth of the mesocotyl. Promotion of the coleoptile, however, was not observed.

Application of mixed Na and K nitrates occasioned an immediate growth promotion of coleoptile and leaves in both the presence and absence of CO_2, and also a much less pronounced promotion of the mesocotyl in CO_2; there was no effect in air. This enhanced growth of the coleoptile and leaves was coupled with a correspondingly greater dry weight and also with an increased outflow of reserves from the endosperm into the plumule.

Thus, while externally applied sugars seemed not to reach the coleoptile, those made available from the endosperm as a result of improved nitrogen supply were rapidly translocated to it. Simultaneous provision to the roots of nitrate and sucrose did not improve the absorption and translocation of sugar.

An analysis of covariance has been computed using the mesocotyl and coleoptile length data together with the outflow from the endosperm and the conclusions so derived are discussed in relation to the problem of growth integration in etiolated oat seedlings.

Introduction

THE change in growth behaviour displayed by etiolated oat seedlings when grown in air enriched with carbon dioxide—promotion of the mesocotyl and depression of the coleoptile—is known to be associated with a reduced transfer of reserves from the endosperm to the embryo. Since the majority of this reduced supply is retained by the mesocotyl the restricted growth of the coleoptile might possibly be due to partial starvation (Mer, 1957). This hypothesis has been tested by providing various nutrients in culture solution to the germinating grain.

[Annals of Botany, N.S. Vol. 23, No. 89, 1959.]

Materials and Methods

Avena sativa (Svalöf 'Victory') was used for all experiments. The plants were grown as previously described in closed chambers within light-tight tin boxes. The chambers were continuously swept with air, which after purification by passage through an activated-carbon filter was saturated with water vapour. Into this air-stream carbon dioxide was injected from a cylinder to give a concentration of 5 per cent. (Mer, 1953).

London tap-water was the normal culture medium and to this was added in different experiments either carbohydrate (at a concentration of 20 g./l.) and/or the mixed nitrates of K and Na, or Ca and Mg (concentration of 4·0 g./l.).

It was not possible to employ sterile growing conditions and, to delay the incidence of bacterial and fungal infection, 200 units of Na penicillin (Glaxo) were added per ml. to the carbohydrate solutions while the seeds themselves were dusted with Spergon. Contamination was rarely observed.

Measurements of coleoptile, mesocotyl, and longest root were taken on separate daily samples (which were either discarded or retained for further analyses) from the 3rd to the 8th day after planting the seed.

Experimental Results

1. *The Effect of Penicillin*

In Table 1 is recorded the result of one of the experiments performed to examine the effect of penicillin on the growth of oat seedlings. The data show that root growth was significantly reduced but that extension of the mesocotyl was not affected. Coleoptile growth was initially unchanged, but the 7th- and 8th-day samples showed significant reductions. Krasilnikov (quoted by Crowdy and Pramer) has detected penicillin in all parts of cress, maize and

Table 1

The Effect of Na-Penicillin (200 units/ml.) on the Growth of Etiolated Oat Seedlings in the Presence and Absence of Carbon Dioxide

Mean length of 16 replicates per sample. Variability quoted is the standard error of the mean

	Day	3	4	5	6	7	8
				Mesocotyls			
$+CO_2$	+Na-P	8·3±0·64	47·3±1·81	79·3±2·32	95·9±2·79	112·3±2·73	118·6±2·75
	−Na-P	12·4±1·13	46·0±1·77	83·2±1·95	97·6±2·32	116·8±2·79	112·3±2·06
$-CO_2$	+Na-P	24·3±1·42	51·5±1·94	68·7±1·75	69·9±2·06	77·7±2·18	74·2±1·79
	−Na-P	25·8±1·79	56·3±1·20	72·3±1·86	69·2±1·14	76·9±1·14	74·3±2·26
				Coleoptiles			
$+CO_2$	+Na-P	6·3±0·31	11·4±0·60	19·0±0·98	25·8±2·40	48·7±3·11	63·8±4·36
	−Na-P	6·9±0·22	10·9±0·40	19·5±0·89	27·8±1·39	53·7±2·41	71·6±4·08
$-CO_2$	+Na-P	9·3±0·42	14·5±1·03	31·3±2·28	57·3±2·34	62·9±2·36	68·9±2·14
	−Na-P	8·9±0·35	18·4±0·83	31·4±1·64	57·4±2·33	70·4±2·46	77·8±2·34
				Roots			
$+CO_2$	+Na-P	44·1±1·73	60·9±3·19	81·6±4·92	73·1±7·83	99·6±5·72	85·3±4·43
	−Na-P	50·8±2·08	69·4±2·98	95·9±2·69	109·2±3·91	120·0±7·50	127·3±5·53
$-CO_2$	+Na-P	49·8±2·64	60·5±3·53	83·4±4·05	110·1±4·41	99·6±5·56	120·3±6·09
	−Na-P	52·6±2·14	85·6±2·54	103·7±4·45	120·6±3·73	139·0±4·08	155·6±5·79

pea plants after administration through their roots, and in the absence of effects on the mesocotyl this depression of the coleoptile may be regarded as evidence of translocation. No direct tests of entry have, however, been carried out.

Since penicillin was uniformly administered to all treatments and the effects due to carbohydrates and nitrogen, later to be described, became apparent in the early stage of growth, confusion of these effects with that of penicillin is remote.

2. *The Effect of Carbohydrate Supply*

The esult of adding glucose, sucrose or mannitol to tap-water is recorded in Table 2. It will be seen that each carbohydrate slightly reduced mesocotyl growth up to the 3rd day, but that promotion occurred thereafter. The coleoptile behaved reciprocally—its growth was further reduced, in the presence of CO_2 throughout the duration of the experiment, but in the absence of CO_2 only transitorily; there was no effect from the 6th day onwards.

Root growth was depressed by all three carbohydrates (Stenlid, 1957).

TABLE 2

The Effect of Glucose, Sucrose and Mannitol (2 per cent.) on the Growth of Etiolated Oat Seedlings in the Presence and Absence of Carbon Dioxide

Means and standard errors of 15 replicates per sample

	Day	3	4	5	6	7	8
				Mesocotyls			
$+CO_2$	G	24·8±0·86	56·9±1·32	78·5±1·99	98·4±2·10	110·5±2·85	106·7±3·61
	S	26·2±1·20	57·1±1·37	80·7±1·47	100·3±1·55	101·8±2·04	100·5±2·29
	M	24·5±1·06	55·2±1·40	81·7±1·82	102·1±2·15	108·8±3·17	105·1±2·85
	Controls	29·2±1·52	59·3±1·93	73·0±1·99	89·3±1·61	82·4±2·19	80·9±2·15
$-CO_2$	G	32·6±0·92	53·0±1·67	70·2±1·79	67·5±1·33	59·3±2·74	60·5±1·61
	S	34·1±0·99	56·0±1·22	64·1±1·97	61·1±1·98	58·8±1·70	64·1±2·58
	M	31·1±1·10	54·0±1·19	67·7±1·41	70·3±2·28	60·1±2·02	64·1±2·72
	Controls	35·9±1·02	48·1±1·23	55·4±1·66	61·1±1·20	51·7±2·20	53·7±1·61
				Coleoptiles			
$+CO_2$	G	9·7±0·27	14·4±0·67	23·8±0·89	31·5±1·81	52·7±2·86	64·1±2·51
	S	10·4±0·42	16·0±0·49	24·8±1·18	33·5±1·70	57·6±2·75	65·4±1·96
	M	10·1±0·31	15·1±0·76	23·6±1·15	35·1±1·80	51·7±2·95	60·1±2·95
	Controls	12·3±0·47	18·9±0·71	35·0±2·07	45·5±2·28	66·8±2·57	71·9±2·09
$-CO_2$	G	11·4±0·58	25·4±1·66	38·1±1·27	52·7±2·09	62·7±1·51	64·1±1·24
	S	13·3±0·45	24·9±1·20	41·1±1·56	56·2±1·72	62·4±1·40	65·3±1·01
	M	12·5±0·68	25·1±1·36	37·8±1·33	52·5±2·04	61·9±1·48	65·6±1·56
	Controls	13·4±0·69	36·1±1·04	47·9±1·72	59·3±1·67	63·4±1·66	65·3±1·18
				Roots			
$+CO_2$	G	52·5±1·91	73·6±2·81	86·6±2·57	106·9±3·45	114·9±3·05	125·9±4·55
	S	57·9±1·27	74·3±1·21	85·0±2·74	85·1±3·01	101·3±3·57	106·0±4·58
	M	57·6±1·61	75·8±2·01	99·3±2·47	110·3±4·57	127·7±4·54	128·7±3·73
	Controls	58·5±1·52	79·9±1·52	102·2±4·07	119·1±4·04	131·2±6·07	137·1±5·60
$-CO_2$	G	56·9±2·36	68·7±2·92	90·3±2·63	90·0±3·68	110·7±5·16	145·8±5·50
	S	54·9±1·48	62·8±2·14	75·4±1·83	75·9±3·07	73·5±1·82	99·1±3·69
	M	48·5±2·83	71·5±3·43	90·6±2·80	93·9±2·63	135·8±4·91	137·5±6·46
	Controls	50·9±2·34	78·8±2·06	112·9±2·87	126·8±4·91	151·3±4·15	165·5±5·22

Mannitol was included in these experiments to examine the effect of an allegedly inert carbohydrate. The response of the plants, however, was similar

to that occasioned by glucose and sucrose. Intact seedlings thus appear to metabolize these substances with equal facility. Shibko and Edelman (1957) have recently suggested a role for *d*-mannitol in carbohydrate interconversions.

These results indicated that while mesocotyl growth was proceeding the applied carbohydrate was used locally to promote its growth still further, and that promotion of the coleoptile might only occur if that of the mesocotyl were prematurely terminated. This was done by heating the seedlings in

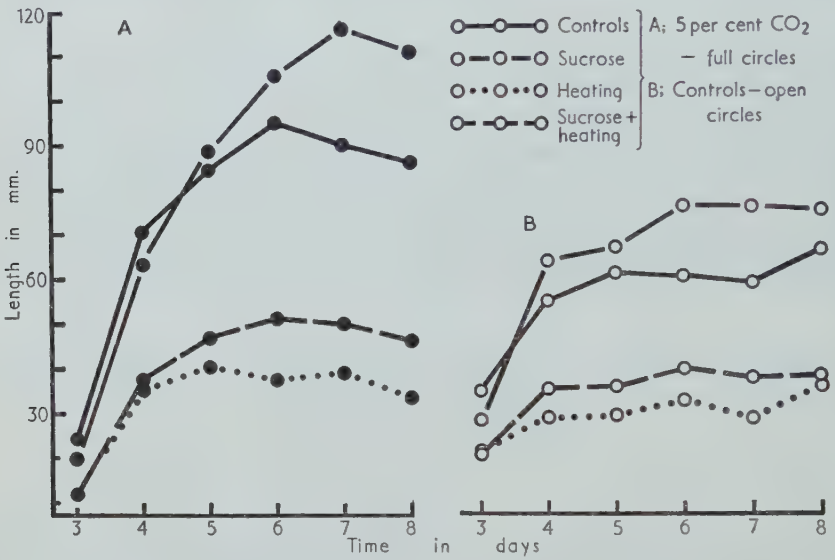


FIG. 1. The effect of sucrose supply and heating on mesocotyl growth in the presence and absence of carbon dioxide.

darkness at 40° C. for 3 hrs. after 2-days' normal growth. The other factors in the experiment were sucrose and carbon dioxide and these were combined with heating to complete a 2^3 factorial arrangement. During heat treatment the air-stream, of necessity, was discontinued, but the plants were supplied with tap-water$\pm$sucrose to correspond with the unheated controls which remained in their respective solutions in the thermostat.

After treatment the plants were returned to their former position within the tins and aeration was resumed. Daily samples were then taken as usual.

The result of one such experiment is presented in Figs. 1, 2, and 3. 1A and 1B show the behaviour of the mesocotyls in carbon dioxide and air respectively. The depressive effect of heating on the growth of the mesocotyl is immediately apparent. Sucrose promoted the growth of these mesocotyls to approximately the same extent in the presence and absence of carbon dioxide.

In Fig. 2A is shown the progress with time of coleoptile growth. The remaining treatments of the factorial design are depicted as differences from these controls, which appear as the straight lines (Figs. 2B and 2C refer to carbon dioxide and air respectively).

Sucrose consistently depressed coleoptile growth while heating caused a transitory growth promotion. When applied together the effect of heating predominated, more so in the presence of CO_2 than in its absence. In general, therefore, the effects of these factors on coleoptile growth were simply the inverse of those shown by the mesocotyl.

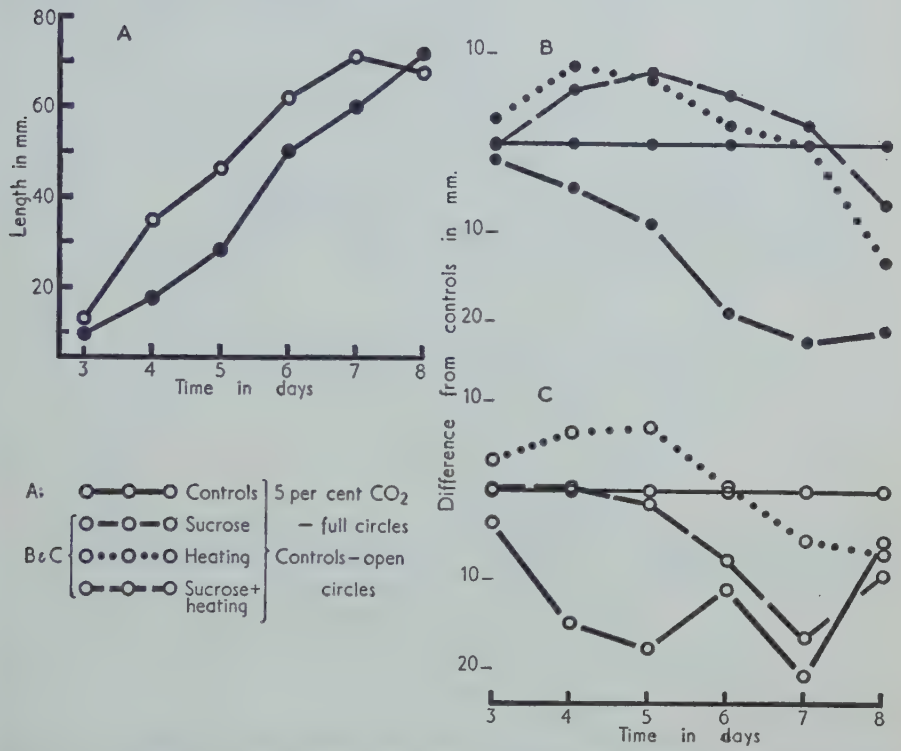


FIG. 2. The effect of sucrose supply and heating on coleoptile growth in the presence and absence of carbon dioxide. In B and C the effects of sucrose supply and heating separately and together are shown as differences from the controls.

The interactions between these factors over the entire growing period cannot be adequately evaluated because a marked correlation occurs between mean length of both mesocotyls and coleoptiles and their corresponding variances. The daily samples were therefore analysed individually and the mean squares are quoted in Table 3; the arrows indicate promotion or depression and the alteration of these effects with time and their significances can be readily seen.

The growth responses of the roots (length of the longest root) are shown in Fig. 3. Sucrose depressed growth while heating was promotive irrespective of treatment with carbon dioxide. These results corroborated previous observations (Mer, 1951) but Choudhury (1957) found that similar heat treatment of rye did not promote root growth. As rye has no mesocotyl,

promotion in *Avena* is probably correlated inversely with the heat-induced reduction of mesocotyl growth.

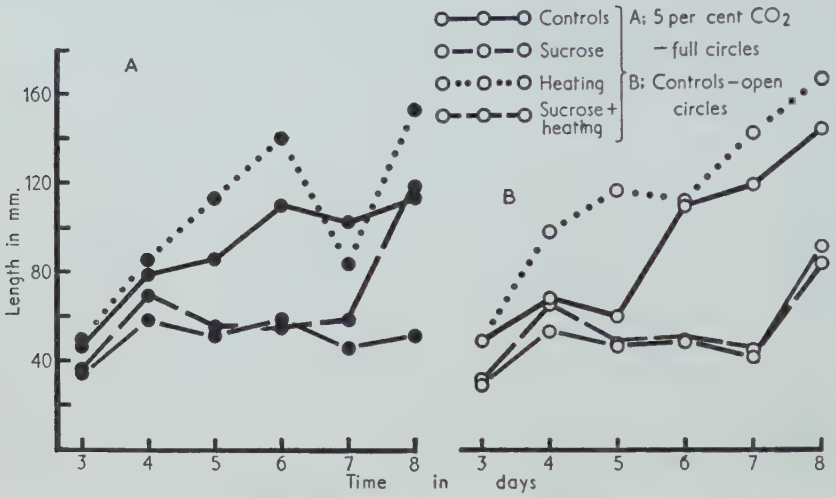


FIG. 3. The effect of sucrose supply and heating on root growth in the presence and absence of carbon dioxide.

TABLE 3

Analyses of Variance of the Daily Samples showing the Mean Squares Attributable to Each of the Factors Carbon Dioxide, Heating, and Sucrose, and their Mutual Interactions

The arrows indicate promotion or depression of the significant effects

Day	3	4	5	6	7	8
			Mesocotyls			
CO_2	2,457·1↓	1,184·4↑	8,823·7↑	12,423·7↑	17,812·0↑	7,426·1↑
Heat	3,131·4↓	24,912·0↓	40,959·0↓	57,334·4↓	61,562·7↓	63,204·3↓
Sucrose	273·0↓	190·0↑	969·0↑	4,071·7↑	6,720·0↑	4,416·5↑
CO_2 × Heat	1·4	91·9↓	1,026·7↓	4,308·0↓	4,889·6↓	4,662·5↓
CO_2 × Sucrose	16·9	775·2↓	3·7	4·4	313·6↑	1,267·5↑
Heat × Sucrose	190·0↑	99·0↑	16·9	60·2	740·0↓	634·8↓
Triple interaction	3·0	273·0↑	6·1	267·0	158·7	61·6
Error	12·4	23·0	36·4	54·2	59·6	83·4
			Coleoptiles			
CO_2	304·0↓	4,953·7↓	5,440·5↓	2,980·0↓	891·1↓	8·0
Heat	249·4↑	3,193·0↑	4,272·1↑	1,732·8↑	1,003·4↑	190·0
Sucrose	255·2↓	1,519·4↓	2,236·0↓	2,270·7↓	5,005·2↓	891·1↓
CO_2 × Heat	15·4↓	10·2	1·2	963·3↑	1,235·2↑	267·0
CO_2 × Sucrose	5·2	330·0↑	607·5↑	28·0	267·0	85·0
Heat × Sucrose	1·0	226·9↑	662·7↑	1,178·1↑	2,193·1↑	1,695·0↑
Triple interaction	4·4	72·1↓	0·9	710·5↑	414·4↑	1,110·2↑
Error	3·7	17·4	45·5	65·6	86·2	110·7

The principal result of this experiment, however, was that the device of curtailing mesocotyl growth in order to facilitate transfer of the absorbed carbohydrate to the coleoptile failed in its purpose; promotion of coleoptile

growth due to the application of sucrose in the presence of carbon dioxide was not observed.

3. *The Effect of Nitrogen—as Nitrate*

The addition to tap-water of mixed sodium and potassium nitrates occasioned promotion of coleoptile growth both in the presence and absence of

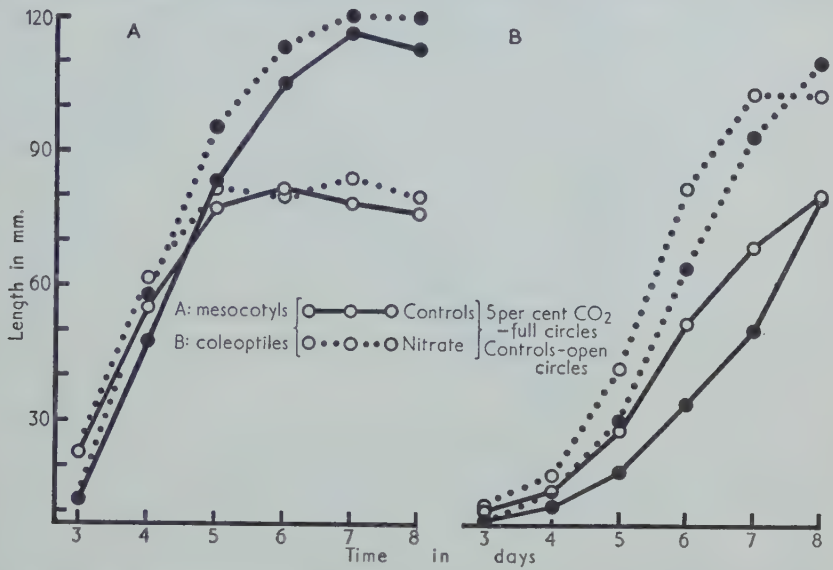


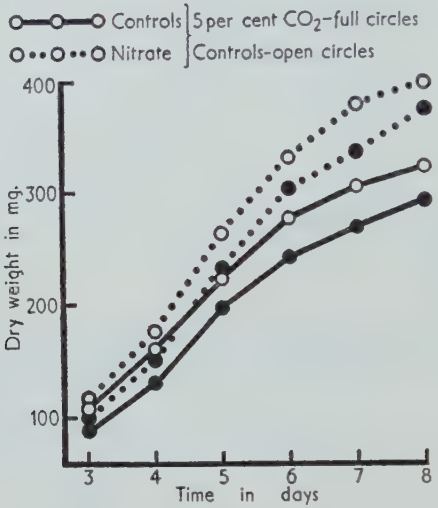
FIG. 4. The effect of nitrate supply on the growth of mesocotyls and coleoptiles.

carbon dioxide. A similar result was also obtained with mixed calcium and magnesium nitrates; further experiments in which nitrate was excluded provided no comparable effect thus indicating that nitrogen supply was the important factor.

To determine whether or not the increased growth of the coleoptile was associated with a corresponding increase in the outflow of reserves from the endosperm an experiment was carried out with mixed Na and K nitrates using the weighed seed technique already fully described (Mer, 1957). In the present experiment two samples (each of 10 plants) were obtained for each of the four treatments ($\pm CO_2$; $\pm 'NO_3$) on six consecutive days. The effects of these treatments on the growth of mesocotyls and coleoptiles are shown in Fig. 4. Nitrogen supply has scarcely any effect on mesocotyls grown in air; in carbon dioxide it is slightly but consistently promotive. The coleoptiles are promoted by nitrogen supply in both the presence and absence of carbon dioxide.

It is not intended to present the fresh-weight and water-content data since these conformed with the observations already published (Mer, 1957). It was, however, noted that nitrogen increased the water content of all parts of the seedlings.

The dry-weight data are shown in Figs. 5 and 6. It will be seen in Fig. 5 that provision of nitrogen increased the total dry weight of the plants irrespective of carbon dioxide treatment, which was always depressive. Supplying nitrogen to the plants treated with carbon dioxide increased their weight so that they were ultimately heavier than the untreated air controls.



IG. 5. The effect of nitrate supply on the total dry weight of the seedlings (2 replicates each of 10 plants).

The data for the mesocotyls (Fig. 6A) showed that the dry weight increases corresponded with the length data, except for the decline in dry weight of the controls which was more pronounced in the presence of nitrate than in its absence.

The dry weights of the coleoptiles agreed very closely with the length data (Fig. 6B).

The leaves showed the largest increment of dry weight due to nitrogen supply (Fig. 6C).

Carbon dioxide generally reduced the dry weight of the roots, and nitrate, too, was somewhat depressive, more so in the controls than in carbon dioxide (Fig. 6D).

It will be seen, as shown in Table 4, that when the data for the individual organs of the seedling were summed for the whole experiment that only the coleoptiles and leaves showed substantial increments in dry weight due to nitrogen supply; the net effect on the mesocotyls and roots was negligible.

The transfer of reserves into the seedling was again determined by estimating for each sample the initial dry weight of the endosperm from a regression and then deducting from this estimate the observed final dry weight.

The regression equation was $y = 4{\cdot}274 + 0{\cdot}870x$, where y is the dry weight of a group of 10 endosperms (with scutellum) and x is the total fresh weight of the 10 grains. The slope (0·870) agrees well with previously observed values

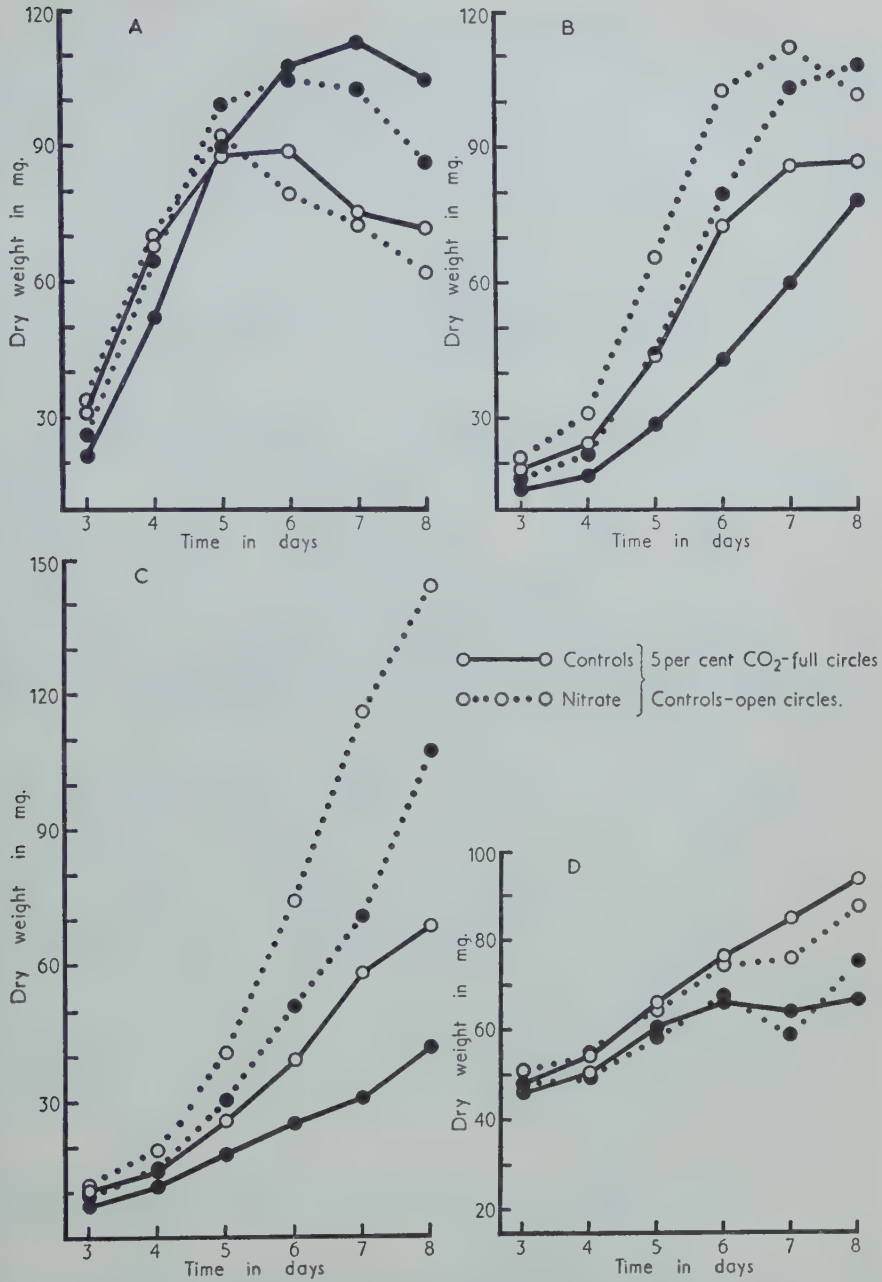


FIG. 6. The effect of nitrate supply on the dry weight of the individual organs of the seedlings.

A—mesocotyls: B—coleoptiles: C—leaves: D—roots.

of 0·866 and 0·868. Similarly, the outflow from the endosperm (Table 5) also agreed with previous observations in showing a consistent reduction due to carbon dioxide. These data further show that application of nitrate promoted transfer of reserves to the seedling both in the presence and absence of carbon dioxide. The analysis of variance shows that the effects of carbon dioxide and nitrate on mesocotyl length, coleoptile length and outflow from the endosperm were all highly significant.

TABLE 4

The Total Dry Weights (in g.), Sums of all the Daily Samples, of the Separate Organs of the Seedlings as Influenced by Carbon Dioxide and Nitrogen Supply

	Controls			Carbon dioxide		
	+N	−N	Difference	+N	−N	Difference
Mesocotyls	0·4120	0·4246	−0·0126	0·4852	0·4902	−0·0050
Coleoptiles	0·4355	0·3333	0·1022	0·3750	0·2423	0·1327
Leaves	0·4059	0·2165	0·1894	0·2786	0·1332	0·1454
Roots	0·4099	0·4251	−0·0152	0·3581	0·3557	0·0024

Thus the two factors employed in this experiment simultaneously affected both extension growth and the transfer of reserves; variation in the latter can, however, be allowed for by covariance analysis thus permitting estimation of direct effects, if any, on extension growth alone. The relationships between outflow and length of plumule (mesocotyl+coleoptile) and mesocotyls and coleoptiles separately are shown in Figs. 7 and 8 respectively.

The relationship for total plumule length is satisfactorily linear although the slopes in the presence and absence of carbon dioxide are different (10·9 and 7·7). That for the mesocotyl (Fig. 8A) is at first linear but later bifurcates corresponding to $\pm CO_2$ while that for the coleoptiles is curvilinear (Fig. 8B).

The result of the covariance analysis (assuming linearity) is quoted in Table 5. It will be seen that the effect of nitrogen supply on mesocotyl elongation has been eliminated, leaving an effect of carbon dioxide and a significant interaction with nitrate. The coleoptiles respond inversely, the carbon dioxide effect has disappeared, but nitrogen induced a highly significant growth promotion. Thus the reduced growth of the coleoptile in the presence of CO_2 may be attributed to a reduced transfer of reserves from the endosperm.

These conclusions will be referred to in the discussion in connexion with Van Overbeek's hypothesis on the growth-correlating mechanism in the etiolated seedling and it is desirable to ensure that they are valid and do not arise from the assumption of linearity.

It is evident that no single curve will accurately fit the two diverging branches of the mesocotyl data. Analyses of covariance have therefore been computed separately on 3 sub-groups: the initial group (up to 150 mg. outflow) and the two divergent groups. In no instance was there a significant effect of nitrogen on mesocotyl length.

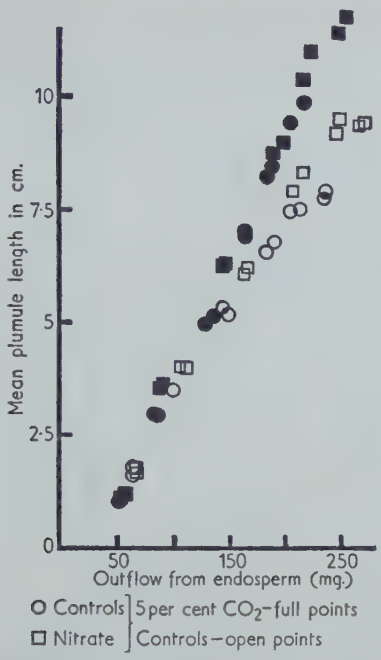


FIG. 7. The relationship between mean length of the plumule (mesocotyl + coleoptile) in cm. and outflow from the endosperm.

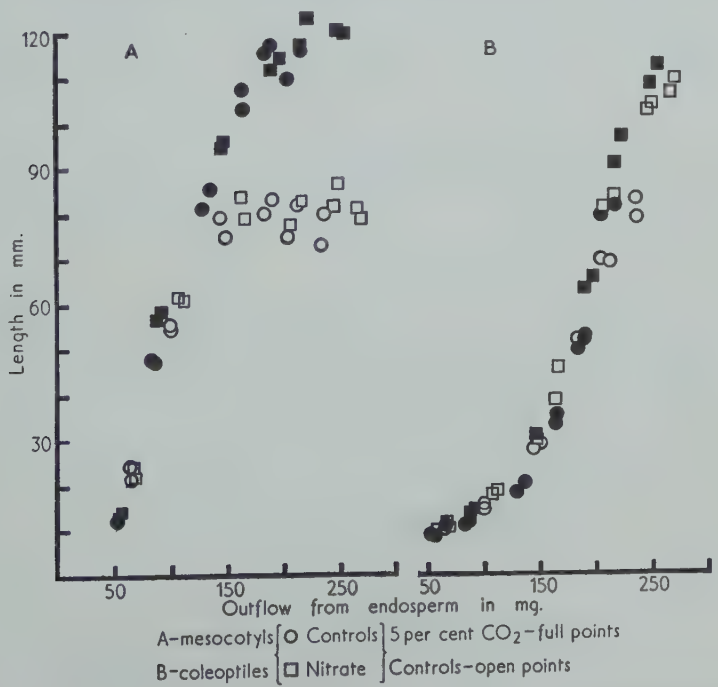


FIG. 8. The relationship between mean length of mesocotyls and coleoptiles in mm. and outflow from the endosperm.

Table 5

Outflow of Reserves from the Endosperm on Successive Days in mg. with Corresponding Mesocotyl and Coleoptile Length in mm.

The entries are the totals of the two samples (each of 10 plants) for each treatment

	$+CO_2$						$-CO_2$					
	$+NO_3$			$-NO_3$			$+NO_3$			$-NO_3$		
Day	Outflow	Mesocotyl length	Coleoptile length	Outflow	Mesocotyl length	Coleoptile length	Outflow	Mesocotyl length	Coleoptile length	Outflow	Mesocotyl length	Coleoptile length
3	109·1	272	185	105·2	254	163	132·9	460	224	127·8	457	208
4	177·9	1,159	282	167·6	956	227	217·1	1,235	367	197·4	1,106	294
5	290·7	1,910	612	262·4	1,672	382	327·2	1,633	842	291·1	1,545	566
6	384·3	2,270	1,292	326·9	2,110	686	419·8	1,608	1,653	371·2	1,637	1,045
7	435·6	2,414	1,884	371·7	2,333	1,078	491·2	1,687	2,077	414·2	1,572	1,389
8	500·0	2,410	2,220	417·7	2,267	1,612	533·0	1,607	2,171	468·8	1,532	1,623
	1,897·6	10,435	6,475	1,651·5	9,592	4,088	2,121·2	8,230	7,334	1,870·5	7,849	5,125

Analysis of Variance

		Mean squares		
	D.F.	Mesocotyls	Coleoptiles	Outflow
Carbon dioxide	1	324,723†	74,892†	4,081†
Nitrate	1	31,212†	440,067†	5,142†
Interaction	1	4,446†	660	0·4
Error	24	713	364	14·6

Analysis of Covariance

		Mean squares		
	D.F.	Mesocotyls	Coleoptiles	Outflow
Carbon dioxide	1	54,818†	495	—
Nitrate	1	914	12,600†	—
Interaction	1	4,815†	767	—
Error	23	484	239	—

† Significance; $P = 0{\cdot}01$

The coleoptile data may be subdivided (at 140 mg. outflow) into two groups having linear regressions and the effect of carbon dioxide did not reach significance in either group. It seems not unreasonable to assume, therefore, that the analysis of covariance quoted in Table 5 provides an appropriate assessment of the data.

It may also be noted that the initial steep part of the relationship between mesocotyl length and outflow (up to 150 mg.; Fig. 8A) has a slope of 7·13, while that for the latter part of the coleoptile data (above 150 mg.) is 7·26 (Fig. 8B). Thus when the mesocotyl was elongating rapidly inflowing nutrients were absorbed by it and coleoptile growth was slow, but when cessation of mesocotyl growth eliminated the demand for nutrients these were transferred to the coleoptile which then grew at virtually the same rate per unit of nutrient supplied as the mesocotyl did previously.

It was now of interest to determine whether nitrate supply would affect absorption of externally applied sucrose in the same way as it influenced the movement of endogenous reserves. The result of an experiment in which nitrate and sucrose were supplied together with carbon dioxide in a 2^3 factorial arrangement is recorded in Fig. 9.

The effects of nitrate and sucrose supply individually were similar to those already recorded. It is evident that for the control mesocotyls the effect of providing nitrate and sucrose together was scarcely different from the effect of sucrose alone, whereas in carbon dioxide growth up to 5 days was similar to the effect of nitrogen alone and then the promotive effect of sucrose became apparent. These factors thus seem to behave independently and no interaction between nitrate and sucrose appeared in the analyses of variance of the daily samples.

The effect of sucrose on coleoptile growth in the presence of nitrate was uniformly to depress growth as compared with the effect of nitrogen alone, which again was promotive: independent action is again indicated and this was confirmed by the absence of interactions in the analyses of variance.

Discussion

The hypothesis under examination in this work attributes to shortage of nutrients the reduced growth shown by the coleoptiles of oat seedlings which have been exposed to high CO_2 concentrations. The nutrient shortage arises from two causes—a reduction in outflow from the endosperm and the utilization of a greater proportion of this reduced supply by the mesocotyl of which growth is promoted. Consequently provision of the appropriate metabolites by an alternative route, i.e. via the roots, should eliminate the growth reduction; the effective metabolite appears to be nitrogen and not carbohydrate.

The growth responses of the seedlings to externally supplied carbohydrate —promotion of the mesocotyl and further reduction of the coleoptile—raise interesting issues, however. Sucrose invariably promotes the growth of isolated coleoptile segments when it is supplied to them in the external medium, and the reason for its failure similarly to promote the growth of coleoptiles of

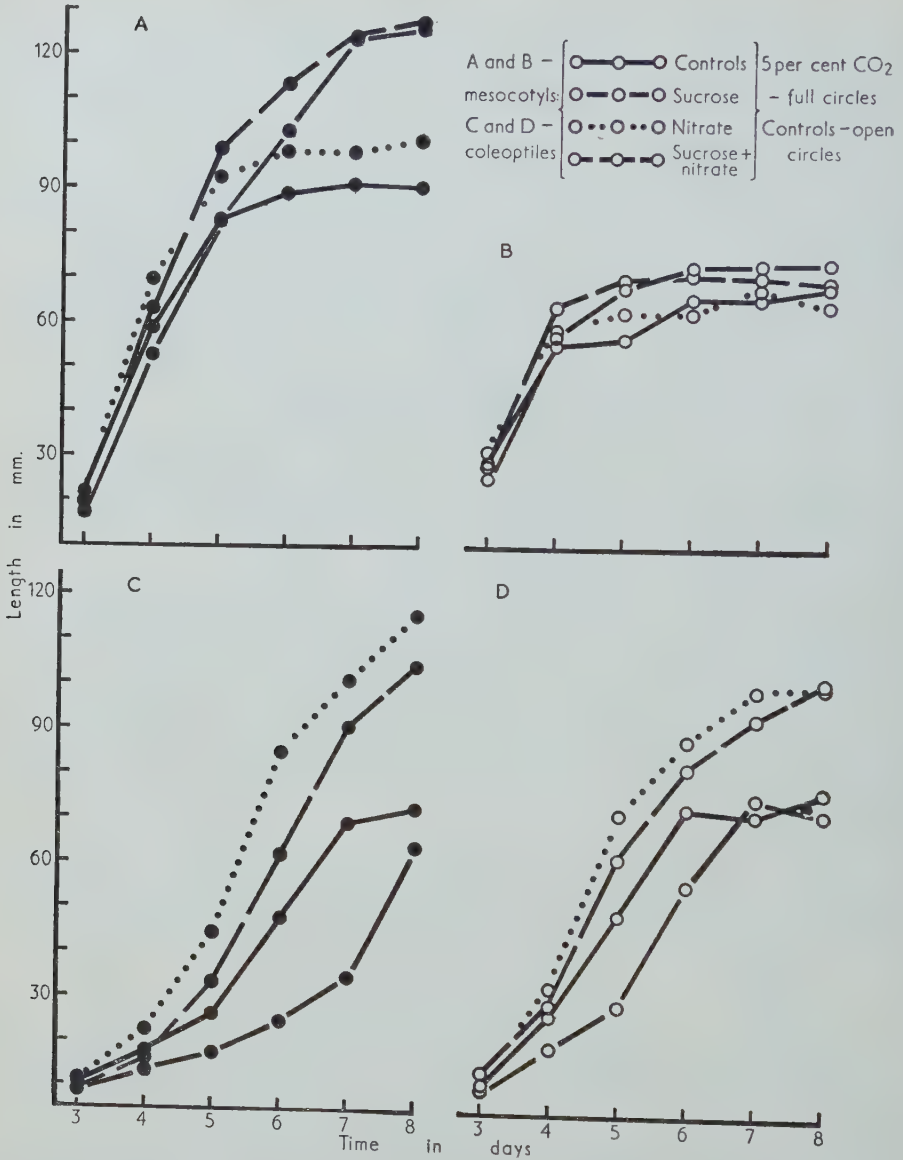


FIG. 9. The effect of nitrate and sucrose supply on the growth of mesocotyls and coleoptiles in the presence and absence of carbon dioxide.

intact seedlings needs examination. The sugar is absorbed and transported at least as far as the mesocotyl, for this shows the usual response—a growth promotion. It therefore seems that the sugar does not reach the coleoptile, and its reduced growth must be a consequence of the promotion of the mesocotyl rather than a direct effect of sucrose on the coleoptile itself. Thus the statistically significant depressive effects of sucrose on the coleoptiles of intact

seedlings, and also its interaction with heating shown in Table 3, should be regarded as correlative growth phenomena.

Since the growing mesocotyl metabolized the absorbed carbohydrate while it was in transit, it became evident that translocation to the coleoptile might only occur if mesocotyl growth was prevented. This was accomplished by heating the seedlings in darkness. It was found, however, that even under these circumstances externally provided sucrose caused a promotion of the mesocotyl together with the attendant depression of the coleoptile, so that the experiment failed in its purpose. Other methods of stopping mesocotyl growth, for example, red light, remain to be investigated.

The quite different effects of nitrogen (administered as nitrates) on the growth of these seedlings were: (i) a marked promotion of the coleoptile and leaves (Fig. 4B), but only a slight promotion of the mesocotyl in the presence of carbon dioxide (Fig. 4A); (ii) an increased transfer of reserves from the endosperm into the seedling (Table 5); and (iii) a preferential migration of these additional nutrients to the coleoptile and leaves (Table 4 and Fig. 6).

It seems unlikely that the nitrates supplied via the roots would penetrate the endosperm and affect directly the metabolic transformations occurring there. It is much more probable that translocation takes place directly to the coleoptile so that the increased outflow of reserves from the endosperm must be regarded as a secondary effect due to growth processes initiated in the coleoptile and leaves by the additional nitrogen which create a demand for carbohydrate. Also, in view of the promotion of the coleoptiles of the control seedlings when supplied with nitrate (Fig. 4B) it may be inferred that coleoptile growth in intact seedlings is normally limited by nitrogen supply.

Heretofore the depressed growth of the coleoptile under high CO_2 has been referred directly to lack of carbohydrate, but these results suggest that if CO_2 restricted the transfer of nitrogenous metabolites from the endosperm to the plumule then similar variations in the growth of the coleoptile would ensue.

Further, it may be noted that the nutrients transferred from the endosperm to the coleoptile travel through the mesocotyl, whatever its length, without being utilized in transit, thus presenting a situation quite different from that occurring when sucrose is supplied through the roots.

The absence of an effect of nitrogen on mesocotyl growth is noteworthy because its growth is associated with the activity of the nodal meristem (Boyd and Avery, 1936; Hansen, 1957; Tucker, 1957) and since nitrogen is essential for the synthesis of cytoplasm it might have been expected that provision of nitrogen would promote its growth. But it does not appear to do so and it must be inferred that the mesocotyl normally has an optimum nitrogen supply. This may be a consequence of its proximity to the endosperm which allows it to have first claim on the inflowing nutrients.

Since elongation of the coleoptile is due almost entirely to cell extension the promotion resulting from additional nitrogen supply may be referred to enhanced auxin synthesis. Evidently a similar enhancement of auxin synthesis does not occur in the mesocotyl. Alternatively growth promotion of

the coleoptile may be referred to enhanced protein synthesis which is known to be associated with cell extension (Robinson and Brown, 1952: Avery and Engel, 1954). Unpublished work has, however, shown that nitrate does not promote the growth either of isolated coleoptile segments or of whole coleoptiles severed from seedlings so that the presence of the coleoptile tip is not the decisive feature. The important factor appears to be the form in which the nitrogen is supplied to the coleoptile.

Attention may now be directed to more general aspects of seedling growth. It may be recalled that the investigation of the physiology of etiolated oat seedlings of which this is part was undertaken to elucidate certain inconsistencies in Van Overbeek's hypothesis (1935, 1936) attributing the control of mesocotyl growth to the auxin-producing tip of the coleoptile. Considerable doubt has been thrown on the validity of the theory (Mer, 1951) and further doubt is cast by the effect of CO_2 on these plants.

If it were true that the integration of plumular growth was accomplished by auxin flowing downwards from the coleoptile tip then the reduced growth of the coleoptile under high CO_2 conditions must be interpreted as signifying a correspondingly reduced amount of auxin. If so, then the promotion of the mesocotyl becomes unaccountable.

If, however, it be assumed that CO_2 treatment occasions no change in auxin content then reduced extension of the coleoptile consuming proportionately less auxin would leave a larger quantity available for the mesocotyl—hence a growth promotion. The elongation of the coleoptile would thus ultimately be conditioned by some factor other than auxin, a conclusion at variance with Van Overbeek's hypothesis but consistent with Went's thesis of two contributing factors—auxin flowing downwards and a 'food factor' flowing upwards from the endosperm.

If total length be regarded as a measure of auxin it will be seen in Table 5 (and Fig. 7) that carbon dioxide appears to promote auxin synthesis, for total plumule length is 32,474 mm. in its presence and only 26,654 mm. in its absence, and this additional length is associated with less 'food factor'. Furthermore, the two stream hypothesis still requires a downward flow of auxin from the coleoptile tip, which as already mentioned, has been queried, but only by results derived from wounded plants. The present data, derived from intact plants, furnish the following arguments, which contribute to the elucidation of the situation: (*a*) promotion of the mesocotyl under high CO_2 concentrations cannot be a consequence of the reduced growth of the coleoptile because administration of nitrate gives rise to plants having not only longer mesocotyls, but they similarly have long coleoptiles; as shown in Fig. 4B they exceed in length those of the aerated control plants; (*b*) the rapid growth of the coleoptile induced by application of nitrate indicates the presence of an ample supply of auxin, either present and not utilized or synthesized from the inflowing metabolites. But none of this auxin reaches the mesocotyl for, as shown by the analysis of covariance (Table 5), the slight extra growth shown by the mesocotyl as a consequence of supplying

NO_3 may be attributed to the additional reserves made available from the endosperm; (*c*) the analysis of covariance further shows that after allowing for growth variation attributable to the differing amounts of reserves supplied by the endosperm, there remains a highly significant effect of CO_2 on mesocotyl growth but no such effect on that of the coleoptile. Thus promotion of the mesocotyl cannot result from a transfer to it of a metabolite formed in the coleoptile by treatment with CO_2.

It would thus appear that some factor induced by treatment with CO_2 leads to the rapid growth of the mesocotyl and this is coupled with the retention of the inflowing metabolites, which in consequence deprives the coleoptile of these nutrients. Nitrogen supply is not the factor concerned. A similar sequence of events might apply to the coleoptile but connected with nitrogen metabolism; the growth so initiated being again associated with an accumulation of carbohydrate flowing in from the endosperm.

These data together with the derivative arguments are manifestly inconsistent with Van Overbeek's hypothesis, which may now reasonably be revised.

In darkness mesocotyl growth in *Avena sativa* takes precedence over that of the coleoptile and it proceeds independently of the auxin metabolism of the coleoptile. When the extension growth of the mesocotyl begins to decline elongation of the coleoptile is accelerated. Treatments which promote mesocotyl growth in the absence of an ample nitrogen supply simultaneously reduce growth of the coleoptile by depriving it of metabolites. When nitrogen is abundant growth of the coleoptile is less dependent on that of the mesocotyl.

These experiments have thus thrown some light on the correlative growth phenomena shown by etiolated oat seedlings, but the specific effect of carbon dioxide on mesocotyl extension still remains to be elucidated. However, the overriding importance of nitrogen for the growth of the coleoptile has been indicated. The inverse correlation between coleoptile and mesocotyl growth seen in seedlings grown without an external nitrogen supply can be modified by external supply of this nutrient in such a way that rapid growth of mesocotyl and coleoptile occurs simultaneously, and that under these conditions the depressive effect of CO_2 on coleoptile growth is no longer observed.

The analysis of covariance (Table 5) has indicated that the effect of carbon dioxide on the seedling is confined to the mesocotyl but that a subsidiary effect is exerted on the endosperm leading in some way to less adequate mobilization of its reserves.

Acknowledgements

I wish to acknowledge the generous help and encouragement afforded me by Professor F. G. Gregory, F.R.S., and thanks are also due to Miss M. K. Baker for her excellent technical assistance.

Literature Cited

Avery, G. A., and Engel, F., 1954: Total Nitrogen in Relation to Age and Position of Cells in *Avena* Coleoptiles. Amer. J. Bot., **41,** 310.

Boyd, L., and Avery, G. S., 1936: Grass Seedling Anatomy; the First Internode of *Avena* and *Triticum*. Bot. Gaz., **97,** 765.

Choudhury, S. H., 1957: Ph.D. Thesis. University of London.

Crowdy, S. H., and Pramer, D., 1955: Movements of Antibiotics in Higher Plants. Chemistry and Industry, **160.**

Hansen, A., 1957: The Expression of the Gene Dwarf-1 during the Development of the Seedling Shoot in Maize. Amer. J. Bot., **44,** 381.

Mer, C. L., 1951: A Critical Study of the Auxin Theory of Growth Regulation in the Mesocotyl of *Avena sativa*. Ann. Bot., N.S., **15,** 179.

—— 1953: An Examination of the Factors affecting Variability in the Growth of the Mesocotyl and Coleoptile of Etiolated Oat Seedlings. Ibid., **17,** 569.

—— 1957: Further Observations on the Effect of Carbon Dioxide on the Growth of Etiolated *Avena* Seedlings. Ibid., **21,** 13.

Overbeek, J. van, 1936: Growth Hormone and Mesocotyl Growth. Rec. Trav. Bot. Neerl., **33,** 333.

—— 1935: On the Growth Hormone and the Dwarf Type of Growth in Corn. Proc. Nat. Acad. Sci. Washington, **21,** 292.

Robinson, E., and Brown, R., 1954: Enzyme Changes in Relation to Cell Growth in Excised Root Tissues. J. Exp. Bot., **5,** 71.

Shibko, S., and Edelman, J., 1957: Randomisation of the Carbon Atoms in Glucose and Fructose during their Metabolism in Barley Seedlings. Biochim. Biophys. Acta, **25,** 642.

Stenlid, G., 1957: A Comparison of the Toxic Effects of some Sugars upon Growth and Chloride Accumulation in Young Wheat Roots. Physiol. Pl., **10,** 807.

Tucker, S. C., 1957: Ontogeny of the Etiolated Seedling Mesocotyl of *Zea Mays*. Bot. Gaz., **118,** 160.

An Optimum Wind Speed for Plant Growth

BY

R. M. WADSWORTH

(*Botany Department, University of Reading*)

With one Figure in the Text

Abstract

Observations on the growth-rate of stands of young plants of *Brassica napus* in a wind tunnel show that relative growth-rate rises with wind speed at low wind speeds but falls again when wind speed is further increased. A wind speed of 0·3 m./sec. caused optimal growth in these experiments. Changes in relative growth-rate were small.

Introduction

STUDY of the effect of wind on the growth of plants has produced two apparently contradictory results:

1. Six authors have examined the dry weight of whole plants after a period of growth in artificial wind (Hill, 1921; Bernbeck, 1924; Finnell, 1928; Martin and Clements, 1935; Rao, 1938; Whitehead, 1957). All found that increase in wind speed *decreased* the amount of growth. They compared the growth obtained in relatively still air with that in wind speeds of between 2·2 and 27 m./sec. among the plants.

2. The results of Deneke (1931) and Heinicke and Hoffman (1933) show an opposite effect of wind: that carbon dioxide uptake and hence assimilation rate *increased* with wind speed. In these experiments leaves or shoots were grown in wind speeds equivalent to about 0·0005 to 0·9 m./sec.

Thus in high to moderate wind speeds, as used in the first group of experiments, higher wind speeds reduced growth whilst in low wind speeds, as used in the second group of experiments, higher wind speeds increased growth. An optimum wind speed for growth would therefore reconcile these results.

Experiments have been undertaken in this laboratory to determine growth-rates in a range of medium to low wind speeds in an attempt to reconcile the above observations. The plants were grown in a wind tunnel so that the low wind speeds could be controlled. This paper contains a preliminary report on work with *Brassica napus* (rape).

Apparatus and Methods

The horizontal wind tunnel made for this work has been set up on a roof and is subject to the light and other climatic conditions prevailing outside: wind speed only is controlled. The tunnel consists of glass sheets supported on a wooden framework; it has four working sections in series, each being half

[Annals of Botany, N.S. Vol. 23, No. 89, 1959.]

the cross-sectional area of the preceding section. The sections are square in cross-section, the smallest being 21 cm. across. Air is drawn by a fan from the smallest to the largest section, provision being made between working areas to even up disturbances in the air stream caused by plants in the preceding section. The wind speeds produced in the lower halves of the four sections of the tunnel are equivalent to speeds of about 1·2, 2·7, 5·8, and 12 m./sec. at a height of 1 metre. Speed is hardly affected by the outside winds. Air takes about 5 seconds to traverse the tunnel.

Rape has been grown from seed in sand culture and placed in the tunnel either initially or when the first leaf was about 1 cm. long. Stands of plants were grown in tins 14×19 cm. with the plants spaced 3·5 cm. apart. Growth was continued until about the fourth leaf appeared, by which time the plants were beginning to shade one another. The cultures were watered each morning with a volume of nutrient solution equal to three-quarters of the water holding capacity of the sand.

The tins were filled to the rim with sand and were placed in the tunnel so that the sand surface was level with the floor of the tunnel. Four to six tins were placed in each section of the tunnel. Whilst the plants were in the tunnel the wind was continuous except during watering. By turning the tins around and by changing the position of the tins within any one section every day the plants in all the tins in that section were given as near the same position history as possible. Leaf and cotyledon areas were determined by planimeter on blueprints. Dry weights were obtained by drying at about 95° C. and weighing after cooling in a desiccator. Relative growth-rates and net assimilation rates were calculated from the formulae given by Blackman and Wilson (1951). Leaf-area ratio was not constant but the error thus introduced into the net assimilation rate is small.

Experimental Results

It is intended to mention in detail the results of two experiments conducted during late May to July 1957. In the first experiment the plants were grown in the tunnel from seed and one tin of plants from each section was sampled 5, 10, 15, and 20 days after the cotyledons opened. In the second experiment plants which had been raised in the open were placed in the tunnel when the first leaf was rather less than 1 cm. long; sampling of two to three tins from each section was carried out at the start and after 3 and 6 days. Each tin contained an average of 17 plants and these were sampled together for dry weight and leaf area. In both these experiments the same behaviour was observed (Fig. 1):

1. When the plants were small, less than 1 cm. tall, relative growth-rate increased with wind speed.
2. Plants 1 to 4 cm. tall showed an optimum wind speed for growth.
3. The largest plants, 4 to 7 cm. tall, showed a decrease in relative growth-rate as wind speed increased.

Leaves appeared at the same rate in all four sections of the wind tunnel, but as wind speed rose the leaf area ratio fell (Fig. 1*b*). The change in leaf-area ratio was not great and so changes in net assimilation rate were generally

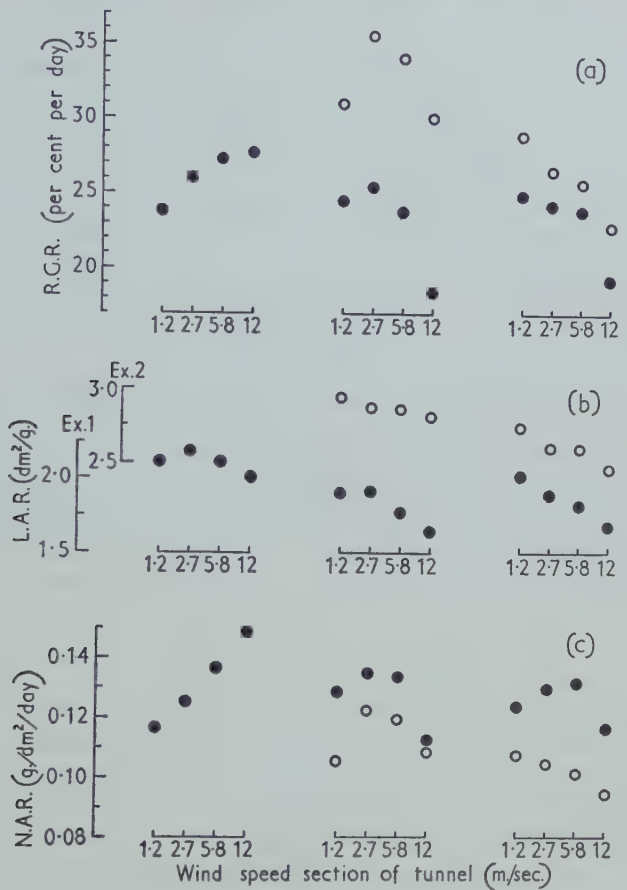


FIG. 1. (*a*) Relative growth-rates, (*b*) leaf-area ratios, and (*c*) net assimilation rates of *Brassica napus* in the four sections of the wind tunnel. The left-hand column of graphs shows results for plants less than 1 cm. tall; the central column for plants 1 to 4 cm. tall; and the right-hand column for plants 4 to 7 cm. tall. The black circles show the results of the first, and the hollow circles the results of the second experiment described.

parallel to those in relative growth-rate (Fig. 1*c*). Analysis of the variance of the results obtained shows that wind speed had a significant effect on relative growth-rate ($P < 0{\cdot}05$, $\sigma = 2{\cdot}2$); and on leaf-area ratio ($P < 0{\cdot}01$, $\sigma = 0{\cdot}09$).

DISCUSSION

In Fig. 1*a*, *b*, or *c* wind speed determines the ratio between the four values in any one graph, but the variation between graphs involves the effect of the prevailing climatic conditions and the age of the plants. Thus in considering

the graphs shown in Fig. 1 the shape of the graphs, but not their magnitude, is comparable.

Friction slows down the air towards the floor of the tunnel; so, as the plants grow taller they grow into a higher wind speed. The graphs in the three height groups may therefore be seen roughly as parts of a single wind speed × growth-rate curve. When the shapes of the graphs shown in the three height groups of Fig. 1*a* (or *c*) are taken together they form a curve showing an optimum wind speed for growth-rate. It is possible that age and time in the tunnel may determine to some extent the plants' response to wind. That the optimum found in these experiments is not caused mainly by these factors is indicated by the graph showing an optimum which is plotted with hollow circles in the middle height group of Fig. 1*a*. This graph is based on the growth during the first three days in the tunnel of plants which were grown outdoors until about 1 cm. tall. These plants have the same age and history and they show an optimum wind speed for growth.

The actual value for the wind speed at the optimum has not been determined accurately, but measurements with smoke among the plants suggest that this lies around 0·3 m./sec. This value is related to the conditions of the experiment. Thus when plants of rape were grown from about 1 cm. tall to about 4 cm. tall in air that was almost water-saturated (90 to 98 per cent. R.H.) significantly greater growth was obtained in the fastest-wind-speed section of the tunnel than in the next to fastest. This indicates a much higher optimum wind speed for growth than that found under the usual experimental conditions.

In previous work in which whole plants have been subjected to wind the amount of growth and not the rate of growth has been measured. Results in this form are difficult to interpret quantitatively because the difference in amount of growth between any two plants, which showed the same initial dry weight, is governed by the duration of growth as well as the rate of growth. Thus the ratio obtained for the amount of growth made by plants subjected to two or more treatments will depend upon the rate and the time of growth.

In my experiments I determined growth-rates, consequently the results are independent of the time for growth. I found that the variation in growth-rate caused by variation in wind speed was not great compared with that produced when other climatic conditions are varied (e.g. light; Blackman and Wilson, 1951). Significantly greater variation in growth-rate is to be expected further away from the optimum, but the wind speeds used in my experiments are in the range normally occurring in the field (Geiger, 1957; Warren Wilson and Wadsworth, 1958).

Acknowledgements

I wish to express my thanks to Professor T. M. Harris, F.R.S., and to Dr. J. Warren Wilson under whose supervision this work was carried out, and to the latter for much helpful criticism during the preparation of this paper.

I am also indebted to the Department of Scientific and Industrial Research for a maintenance allowance during the receipt of which this work was done.

Literature Cited

Bernbeck, O., 1924: Wind und Pflanze. Flora, **117**, 293.

Blackman, G. E., and Wilson, G. L., 1951: An Analysis of the Differential Effects of Light Intensity on the Net Assimilation Rate, Leaf Area Ratio and Relative Growth Rate of Different Species. Ann. Bot., n.s., **15**, 373.

Deneke, H., 1931: Über den Einfluß bewegter Luft auf die Kohlensäureassimilation. Jb. wiss. Bot., **74**, 1.

Finnell, H. H., 1928: The Effect of Wind on Plant Growth. J. Amer. Soc. Agron., **20**, 1206.

Geiger, R., 1957: The Climate near the Ground. Harvard Univ. Press, Cambridge, Mass. pp. 494.

Heinicke, A. J., and Hoffman, M. B., 1933: The Rate of Photosynthesis of Apple Leaves under Natural Conditions. Bull. Cornell agric. Exp. Sta., No. 577. pp. 32.

Hill, L., 1921: The Growth of Seedlings in Wind. Proc. roy. Soc. B, **92**, 28.

Martin, E. V., and Clements, F. E., 1935: Studies on the Effect of Artificial Wind on the Growth and Transpiration of *Helianthus annuus*. Plant Physiol., **10**, 613.

Rao, V. P., 1938: Effect of Artificial Wind on Growth and Transpiration in the Italian Millet, *Setaria italica*. Bull. Torrey bot. Cl., **65**, 229.

Warren Wilson, J., and Wadsworth, R. M., 1958: The Effect of Wind Speed on Assimilation Rate—a Reassessment. Ann. Bot., n.s., **22**, 285.

Whitehead, F. H., 1957: Wind as a Factor in Plant Growth. pp. 84–95 in Control of the Plant Environment. Ed. J. P. Hudson. Butterworths, London.

Verification of the Breeding System in the Bluebell *Endymion nonscriptus* (L.) Garcke

BY

J. YANNEY WILSON[1]

(*Department of Botany, University College of Ghana, Achimota, Ghana*)

Abstract

With due precautions in the handling of pollination experiments, it has been possible to show that the bluebell, *Endymion nonscriptus* (L.) Garcke, is only partially self-sterile.

Introduction

JONES (1950) described the bluebell, *Endymion nonscriptus* (L.) Garcke, as self-fertile, while Elliott (1953) mentioned that from his few trial experiments the species seemed to be self-fertile, although it could equally well be self-sterile; finally, in their recent work on this species, Blackman and Rutter (1954) described the bluebell as self-sterile, and they also stated that it failed to set seed when flowers were enclosed in a bag or bell-jar. Among the methods which the present author considered using to verify the existence of vegetative reproduction in the bluebell (Wilson, 1957, 1958) were some which assumed a self-sterile breeding-system, following Blackman and Rutter. Thus with due precautions to reduce the effect of bagging, experiments were designed in 1955 on the basis of self-sterility, when a cross-pollination among members of a clone should amount to a selfing, and the plants would not be expected to set seed. But the results obtained from this type of experiment were rather contradictory. It therefore became clear that fresh experiments were necessary in the following year (1956) to verify the breeding system itself, and it is those experiments which are discussed here. In addition still more precautions were needed to reduce still further the harmful effects on seed-setting associated with bagging. For this purpose apart from performing some of the experiments in the field others were done in isolated plants in rooms.

Field Pollinations

In the field experiments carried out in Madingley Wood, Cambridge, in 1956 (like the 1955 experiments) the inflorescences were often bagged when none of the flower-buds had opened, and if the lower ones had already opened they were first removed before bagging. The polythene bags used were fastened at their upper end to vertical 2 ft. wooden labels by means of drawing-pins. This kept the plant upright and thus removed or reduced the weight of the bag on the plant. A more important precaution was that the

[1] Formerly of the Botany School, Cambridge.

[Annals of Botany, N.S. Vol. 23, No. 89, 1959.]

bags were removed altogether from some of the plants when nearly all the flowers were withering at the beginning of the seed-setting. This allowed the ovaries to continue development under more normal conditions when no further pollination was possible.

Four plants were selfed and left permanently in bags, and they had an average of 8·15 seeds per fruit; six plants also selfed but later freed from the bags had an average of 10·5 seeds per fruit. With regard to crossed plants, four had the flowers left permanently in the bags and these had an average seed-set of 10·28 per fruit, but the other four in which the bags were later removed had an average of 16·18 seeds per fruit.

These experiments indicate that (*a*) seed was set by both crossing and selfing, (*b*) seed-set was greater by crossing, and (*c*) bagging the plants throughout the fruiting period diminishes the seed-set per fruit.

Room Pollinations

With the room pollinations, some were carried out at room-temperature, while others were done at constant temperatures. At room-temperature (20° C., with average daily fluctuations between 16° and 22° C.) three plants were selfed, and these produced an average of 1·67 seeds per fruit; four were crossed and yielded an average of 8·56. At the constant temperatures of 10° C. four plants were selfed and these gave a mean of 11·44 seeds per fruit; at 15° C. five plants were selfed and gave an average of 6·20. No plants were crossed at these temperatures.

The room-temperature experiments showed a greater seed-set in crossed plants, while the constant temperature ones indicated the effect of higher temperature in reducing the seed-set.

Details of these experiments and of the preliminary pollination experiments in 1955 to verify vegetative reproduction in the bluebell will be published elsewhere.

Conclusions

In conclusion it can be said that bagging has a definitely harmful effect on the development of seed-set in the bluebell, and could even prevent it, and that with adequate precautions as described above, it has been possible to demonstrate that the species is only partially and not entirely self-sterile.

Acknowledgements

The author wishes to thank Dr. H. L. K. Whitehouse under whose guidance this work was carried out, and the authorities of the Low Temperature Research Station and the Botany School, Cambridge, for placing their facilities at my disposal for the work.

Literature Cited

Blackman, G. E., and Rutter, A. J., 1954: *Endymion nonscriptus* (L.) Garcke. J. Ecol., **42**, 629.

ELLIOTT, C. G., 1953: Variation in Chiasma Frequency with Special Reference to the Effect of Temperature. Ph.D. Thesis. Cambridge.

JONES, S. G., 1950: Introduction to Floral Mechanism. Blackie & Son, London.

WILSON, J. Y., 1957: Chiasma Frequency and Other Studies in the Bluebell, *Endymion non-scriptus* (L.) Garcke. Ph.D. Thesis. Cambridge.

—— 1959: Vegetative Reproduction in the Bluebell, *Endymion non-scriptus* (L.) Garcke. New Phytol. (In press.)

Printed in Great Britain at the University Press, Oxford, by Vivian Ridler, Printer to the University